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Studies on the polytene chromosomes of *Chironomus circumdatus* (Diptera : Chironomidae) from Jammu region

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Abstract

Cytogenetic studies were carried out on a population of *Chironomus circumdatus* from Gharana wetland on Indo-Pak border in Jammu region of J&K state This work is the first attempt to examine the polytene chromosomes of this species from this area This species possesses the usual four salivary gland chromosomes with the arm combination BF, DC, EA & G corresponding to the Pseudothummi cytotaxonomic complex. A photomap of polytene chromosome complement (n=4) has been prepared and the diagnostic features in each chromosome have been described

(**Keywords** *Chironomus circumdatus*/ polytene chromosomes/ karyology)

Introduction

The polytene chromosomes of Diptera by virtue of their enormous size have proved to be extremely useful in the study of chromosomal structure and function[1]. Polytene chromosome morphology of chironomids offers a very interesting material for cytogenetic research in view of their distinct banding sequences and characteristic puffing patterns as many closely related species of chironomids do not evidence

distinct morphological differences in the larval stage. Cytogenetic analysis is a promising taxonomic diagnostic tool when combined with morphological characteristics of the separate developmental stages[2]. Based on karyological data, the taxonomic positions of many species were clarified, and a number of new species were described[3,4]. Aside from these benefits cytotaxonomic analysis is able to disclose the phylogenetic relationships among species[3] as well as the path of speciation.

The present study is an attempt to characterize the chromosomes of *Chironomus circumdatus* and prepare a reference photomap of polytene chromosomes. Very little work has so far been done on the genus *Chironomus* from Jammu & Kashmir and this work is the first report on the polytene chromosomes of this species from Jammu region.

Material and Methods

For the present study the larvae of *C. circumdatus* were collected from a water body located in Gharana wetland on the Indo-Pak border in Jammu region of J&K state. Specimens were taken from a fixed location in the central part of the pond on muddy bottom. Polytene chromosomes were fixed and stained according to the conventional squash procedure using 2% Lacto-aceto Orcein[5].

Results and Discussion

The polytene chromosome complement of *C. circumdatus* comprises four synapsed elements, which have been divided into right and left arms. The chromosomes have been marked as I, II, III and IV in order of their decreasing length. The four synapsed elements lie independent of each other due to the absence of chromocenter. Each chromosome has been divided into zones and sections. The division of chromosomes has been done following the method adopted by Martin[6].

The entire complement has been divided into 18 zones, which are numbered and further subdivided into sections by letters. The chromosome arms are marked by letters based on Keyl scheme[3]. Left and right arms of chromosome I are designated as Band F, while those of chromosome II as D and C, chromosome III as E and A., and the right arm of chromosome IV as G, as has been adopted in case of *C. circumdatus* which also belongs to the pseudothummi cytotaxonomic complex. The division of the entire complement into 18 zones is as follows: chromosome I : zones 1-6, zone 1 begins at the free tip of arm B (IL) and zone 6 ends at the free tip of arm F (IR);

chromosome II: zones 7-11, zone 7 begins at the free tip of arm D (IIL) and zone 11 ends at the free tip of arm C (IIR); chromosome III : zones 12-15, zone 12 begins at the free tip of arm E (IIIL) and zone 15 ends at the free tip of arm A (IIIR); chromosome IV : zones 16-18, zone 16 begins at the free tip of arm G (IVR) Diagnostic features of each chromosome have been illustrated in Fig. 1 and the important characteristics are described below.

Chromosome I (BF) :

This is the longest member of the complement. The chromosome is readily identified by a large swelling in 5A. Besides, it is characterized by the presence of some thick (straight or curved) darkly stained bands in sections 1A, 2A, 3C, 4C and 6C Zone 2 exhibits two swellings with a neck-like constriction in between, one is with dark-thick straight and curved bands interspersed with some dark-dotted bands and the other with a few dark bands having light-dotted bands in between. A high degree of asynapsis is observed in arm F in the region 4A and 4B. 5A carries a large swelling with a series of light and dark-dotted bands, which serve as the landmark for the chromosome.

Chromosome II (DC) :

This chromosome is readily identifiable by certain features such as the presence of a large swelling in 8B and some dark, thick bands in 7D, 8A, 8B, 10C, 11A and 11C. The free end of arm C is readily recognizable by the presence of asynapsis in the regions 7A and 7B while in some cells this end is rounded having a few light, thin dotted bands in the beginning of 7A followed by two dark wavy hands. The region 8B is characterized by a large swelling while 8D by a moderate swelling, which can serve as the markers for the chromosome. The regions 10A and 10B show the presence of a heterozygous inversion. Zone 11 at the proximal end of arm C consists of two moderate swellings with a constriction in between and a characteristic fan-shaped terminal end.

Chromosome III (EA) :

The landmark features of this chromosome are the dark-thick bands in 12B, 12D, 13D and 14A, which help, in the easy identification of this chromosome. The expansions at the ends are typical.

Chromosome IV (G).

This is the smallest chromosome of the complement. It is easily recognized by the presence of a large puff in 17 A and a large swelling in sections 17B and 17C. Besides, some dark-thick curved or straight bands in 16A, 16B, 17B, 17C and 18A form other diagnostic features of this chromosome A high degree of asynapsis is observed in all the three zones 16, 17 and 18.

C. circumdatus has good quality polytene chromosomes suitable for a variety of cytological and karyosystematic studies A study of karyotypes is the first stage of comparative karyological analysis, necessary for the discussion of problems of classification, speciation, microevolution and phylogeny The results of the present investigation have been compared with those of the other population of *C circumdatus* from Varanasi[7]. It has been observed that both these populations share a close relationship with respect to the landmark features while the banding pattern in some arms shows certain dissimilarities.

The chromosome I of both Jammu population and Varanasi population show the presence of two swellings in the zone 2 and dark-thick bands in region 3C. The chromosome II of Jammu population exhibits a large and a moderate swelling in 8B and 8D respectively, while the Varanasi population shows small swellings in 8A and 8B. Chromosome III of both the populations shows the presence of thick -dark bands in zone 12 which are of diagnostic importance Chromosome IV of both the populations shows homology with respect to the presence of swellings and puffs

During the present study, the polytene chromosomes of over 53 wild caught larvae of *C. circumdatus* were analyzed for naturally occurring chromosomal polymorphism, which reveals a single incidence of heterozygous inversion in chromosome II in arm C in the regions 10A and 10B. Chromosomal polymorphism due to inversions is basically a method to cope up with the diversity of environment[8,9]. This condition is maintained in nature by balancing selection due to selective advantage of inversion heterozygotes[7]. As a result, many of the inversions have become part of the stable chromosome system in several species of *Chironomus*[10-23]. While the Jammu population of *C. circumdatus* exhibits a single incidence of heterozygous inversion, the Varanasi population shows the presence of seven inversions comprising six paracentric and one pericentric inversion.

Though the results of the chromosomal polymorphism observed in the present case seem to point that the diversity of environment in the wetland due to the accumulation of agricultural wastes ttom nearby fields, animal dung and domestic

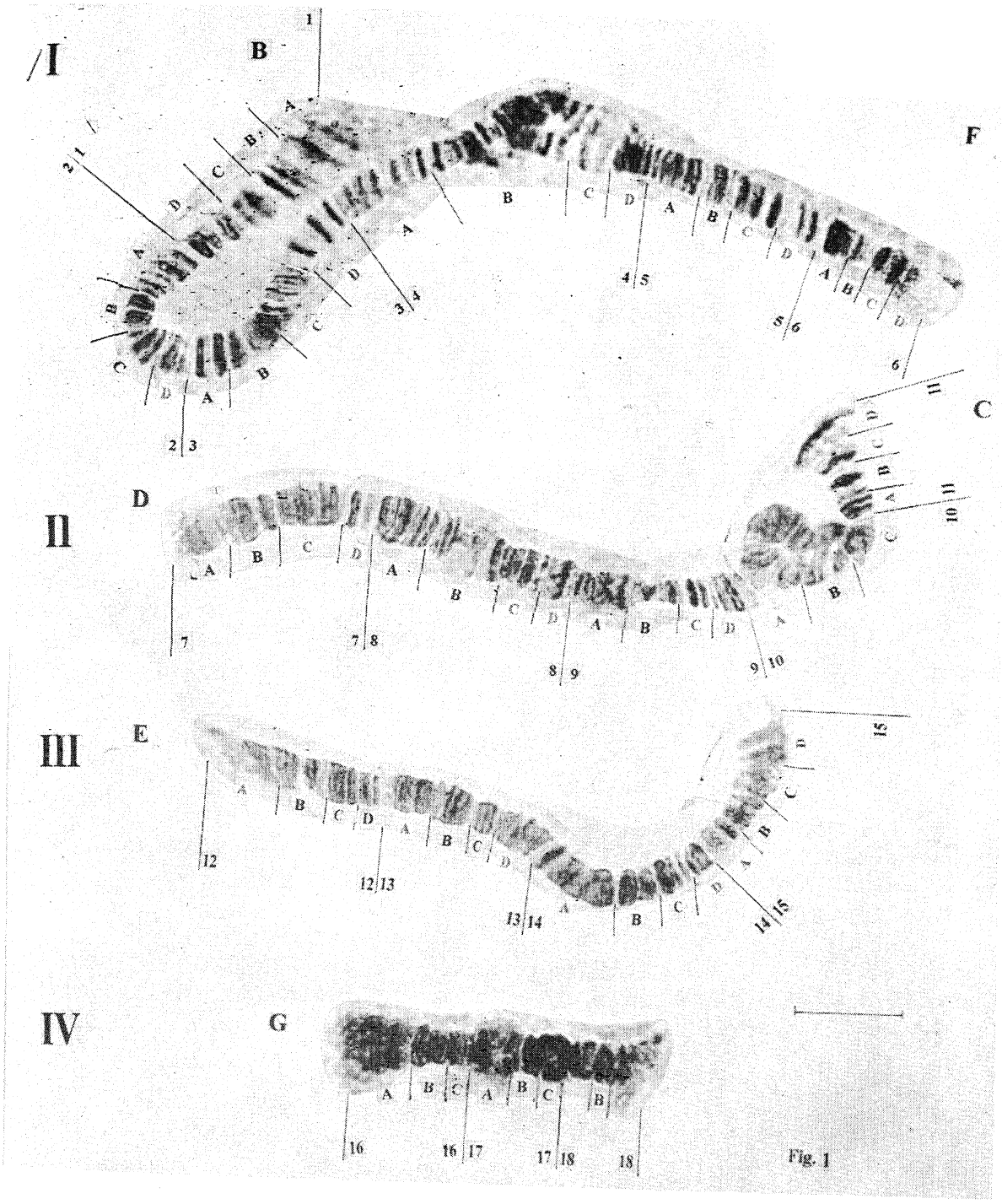

Fig. 1– Photomap of polytene chromosomes of *Chironomus circumdatus*.
Scale=0.01 mm.

wastes into different areas of the pond probably lead to increase in the number of ecological niches and consequently increase in chromosomal variability for adaptiveness, it seems likely that the low degree of polymorphism in the present case enables the Jammu population of *C circumdatus* to occupy a restricted variety of larval habitats. Polytene chromosomal asynapsis as reported in the present investigation depends on small structural heterozygosities as expressed by Beermann[24], which are not visible in the light microscope or to genes which cause localized asynapsis[25]. Further research may provide an insight into the evolutionary relationship amongst the populations found in tropical, sub-tropical and temperate environments.

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Haemolymph withdrawal affects haemocyte count and moulting in plain tiger-butterfly, *Danais chrysippus* L.

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Abstract

Effects of repeated haemolymph withdrawals have been observed on total and differential haemocyte counts in V instar larvae as well as on moulting of both the V larval and pupal instars of *Danais chrysippus* While the present study revealed a marked reduction in total cell count reaching its lowest in prepupal stage, it exhibited a considerable variation in the relative percentage of various cell types The repeated removals of haemolymph affected both the pupal and imaginal moultings and caused reduction in the length of body and wings of adult butterflies

(**Keywords** haemocyte count/haemolymph withdrawal/moulting/*Danais chrysippus)*

Introduction

About four decades after the first report of Jones[1] on differential haemocyte count (DHC) without affecting haemocyte picture in *Rhodnius prolixus* following repeated haemolymph withdrawals, Pandey *et al.*[2] investigated its effect in lemon-butterfly, *Papilio demoleus* and found a reduction in total haemocyte count (THC) along with much variation in relative percentage of various cell types. Because of meagre studies and contradictory earlier reports on one hand, and importance of a tissue like haemocytes in moulting, transport of nutrients and hormones[3,4] and development of immunity among insects[5] on the other, the present study was carried out in *D. chrysippus.*

Materials and Methods

Early larval instars of *D chrysippus* collected from common Ak (*Calotropis gigantea*) plants were raised on fresh leaves in a BOD incubator maintained at 27°C ± 1°C, 75 ± 5% RH and 16:8 hr photophase.[6] V instar larvae and pupae of desired age

groups obtained from laboratory culture were used in present study. To examine the effects of repeated haemolymph withdrawals on THC and DHC, haemolymph was taken out once daily from V instar larvae althrough their development (0-108 hr till prepupa formation) at 24 hourly intervals by puncturing their abdomen with the help of a sterilised sharp needle as described earlier[2]. The oozing haemolymph was drawn either into a thoma blood - cell pipette up to its 0.5 mark and diluted up to 11^{th} mark with Tauber - Yeager's fluid for THC or on a microslide into a thin uniform film for DHC. The wounds were disinfected by sprinkling antibiotic-phenylthiourea (1: 1 w/w) mixture and the larvae were transferred to BOD incubator. In controls, the haemolymph was taken out once from larvae of corresponding age group. To study the effect of repeated haemolymph withdrawals on moulting, V instar larvae on the basis of their body weight were sorted out as early (below 350mg) and late (above 350mg) stage larvae and were subdivided into 3 and 4 groups of 10 larvae each, respectively. Haemolymph from the larvae of all seven groups was drawn either once or several times in a day at definite intervals or once daily throughout larval development. Similarly, the pupae were divided into 2 groups of 10 each having a weight range of 440 ± 25 mg and 485 ± 15mg respectively The number of times and days of withdrawal in pupae were similar to larval groups The volume of haemolymph drawn varied from 25µl-100µl/withdrawal in larvae and only 10µl/withdrawal in pupae of experimental groups Matched mock - punctured larvae and pupae served as controls. In all experimental insects, the haemolymph was taken out from either side of anterior abdominal region to investigate the effect of side of withdrawals, if any, on wing length Measurements of body length and wing - expanse of adults emerging from experimental and control groups of larvae and pupae were made according to the methods described earlier[7,8] The haemolymph from both the experimental and control groups of larvae was pooled and THC was conducted with a standard haemocytometer as per Jones[9]. For permanent preparations, haemolymph smears were made on glass slides that had 2-3 drops of 2% glacial acetic acid which served as an anticoagulant as well as a fixative[10] The smears were stained with 1% Giemsa or observed directly under phase optics[11]. DHC was made by counting at least 200 cells of different categories selected from random areas of stained blood smears of atleast 10 insects from both experimental and control groups and the percentage of different cell types was calculated

Results

The effects of repeated haemolymph withdrawals recorded on THC, DHC and larval-pupal and pupal-imaginal moultings of *D chrysippus* are described as under :

Effect on THC : The haemolymph removal once daily throughout V instar larval development resulted in a gradual decline in THC (Table 1). While much considerable reduction up to 41% was noticed during prepupal stage, only 18% reduction was seen in 48 hr old larvae. However, the cell count remained more or less similar in both the experimental and control larvae, 24hr post -experimentation.

Table 1– Effect of repeated haemolymph withdrawals on THC in V instar larvae of *D chrysippus*. (Values are mean ± SD for 10 insects)

Age (hr) of larvae at the time of haemolymph withdrawal	Body weight (mg)	THC (No of cells/mm^3 of haemolymph)		Approximate per cent reduction in THC
		Experimental	Control	
24	150±20	3295±170	3240±190	–
48	280±30	5460±240	6505±370	18
72	440±25	7535±462	11140±390	33
96	700±15	8560±430	13340±610	36
108 (Prepupa)	680±40	9045±365	15280±670	41

Effect on DHC: A great deal of variation in the percentage of different cell types found in present insect was observed during larval development following repeated haemolymph withdrawals (Table 2). While prohaemocytes (PRs), spherulocytes (SPs) and oenocytoids (OEs) showed an increase in their counts, the plasmatocytes (PLs) and adipohaemocytes (ADs) exhibited a reduction in their number with respect to their controls. The granulocytes (GRs) had a more or less similar count in both the groups. The vermicytes (VEs) appearing for the first time in prepupae also showed a lesser percentage in experimental larvae. A comparison between the relative numbers of a particular cell type in experimental larvae with its control revealed that while the pattern of PR-percentage showed a gradual decline in latter, much variation was seen in experimental larvae. An approximately 2-fold increase in 24 hr and 3-fold increase in 72 and 96 hr old larvae was recorded following repeated removals and this increase in PR-percentage is evidenced by frequent mitotic divisions in them (cf Figs. 1,2). Whereas, the stained smear 24 hr after haemolymph withdrawal showed about 18 per cent PRs in mitotic stage, no such evidence of mitotic division was observed in control insects. The PLs and ADs showing a gradual increase in controls revealed a

pattern quite dissimilar to those of PRs. Likewise, the OEs with a gradual increase in control larvae have shown much variation in their count in experimental group. The most remarkable change of pattern was accomplished by SPs. After showing a similar trend for first two days, they continued to decline in controls but enhanced to the extent of double during 96 to 108 hr in experimental larvae.

Table 2– Effect of repeated haemolymph withdrawals on DHC in V instar larvae of *D chrysippus* (Values are mean ±SD for 10 larvae)

Haemocyte types	Status	Percentage of haemocytes at 24 hr intervals				
		24	48	72	96	108
PRs	Exp	13 0±0 5	7 9±0 7	13 1±1 2	14 1±1 4	6 2±0 2
	Cont	10 3±1 3	4 8±0 8	4 6±0 7	4 6±0 4	4 2±1 2
PLs	Exp	17 2±0 9	22 8±1 7	16 2±1.8	12 5±3 8	22 4±2 8
	Cont	23 6±0 2	29 9±2 6	30 6±1 2	32 1±2 9	38 5±2 3
GRs	Exp	25 4±1 8	23 2±1 7	20 1±0 9	28 2±1 6	21 3±1 4
	Cont	26 0±1 7	24 7±1 9	23 0±0 8	27 3±1 0	19 1±1 1
SPs	Exp	29 0±1 9	28 5±1 4	33 0±1 4	27 3±2 3	28 6±1 7
	Cont	26 6±2 4	24 4±1 2	20 5±1 2	14 2±2 1	13 6±2 1
ADs	Exp	9 3±1 3	11 9±0 9	10 3±2 1	6 3±2 4	8 4±0 8
	Cont	10 8±1 2	12 6±0 8	13 0±1 2	13 1±1 2	14 6±1 2
OEs	Exp	6 1±0 4	5 7±0 04	17 3±2 1	11 6±2 4	11 9±1 2
	Cont	2 7±0 3	3 7±1 0	8 6±1 1	8 7±0 5	8 3±0 8
VEs	Exp	–	–	–	–	1 2±0 5
	Cont	–	–	–	–	1 9±0 6

Effect on Moulting · The repeated removals of haemolymph affected both the pupal as well as imaginal moultings as summarised in Table 3. All early V instar larvae from which the total haemolymph withdrawal exceeded 100 µl died. Even late

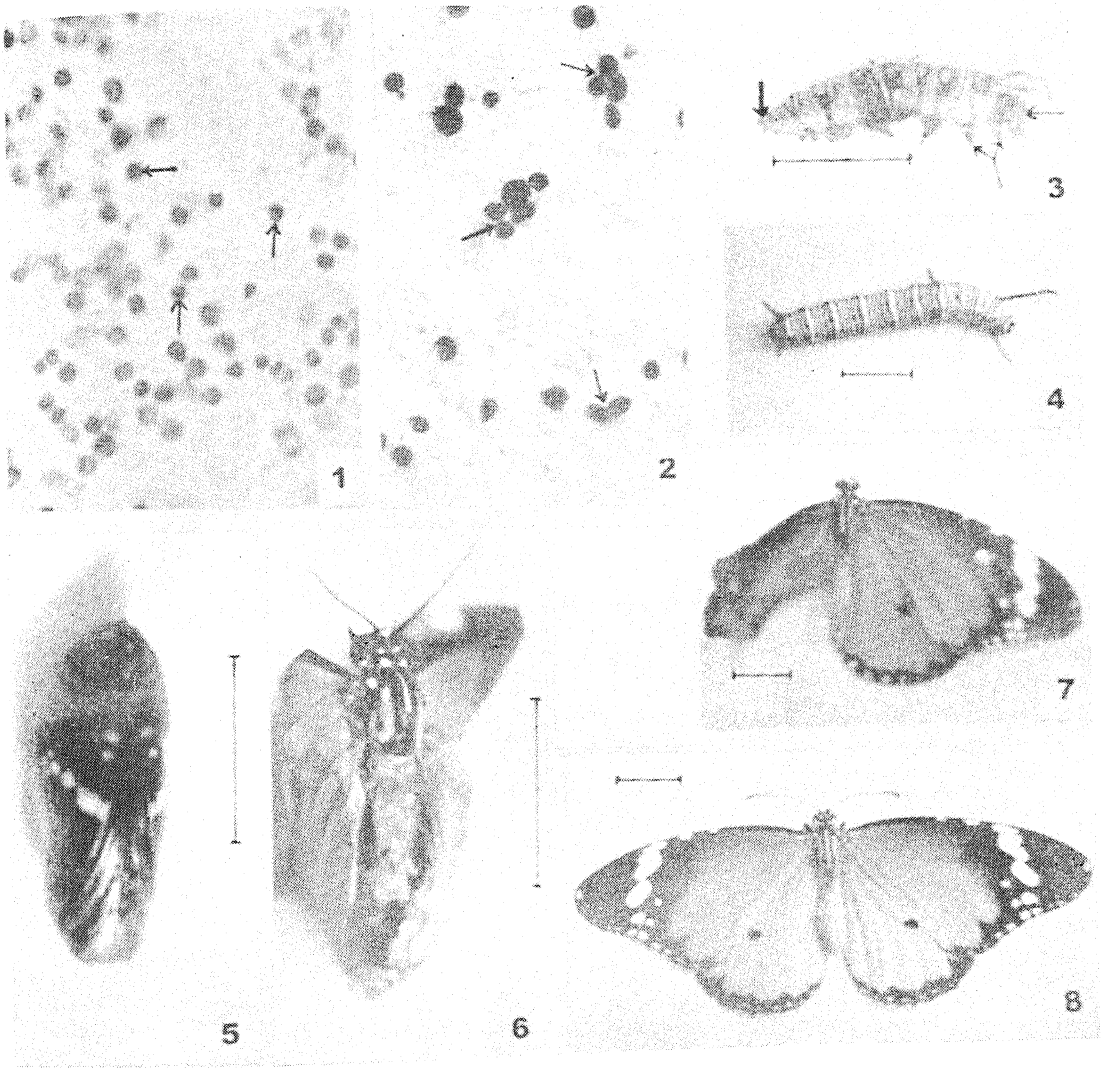

Fig. 1– Blood smear of normal V instar larva showing rounded PRs (arrows) and other cell types x 600. Fig. 2– Blood smear of early V instar larva showing mitotic divisions (arrows) in PRs 24 hr after haemolymph withdrawal x 600. Fig. 3– Larval-pupal intermediate (LPI) with head capsule and thoracic legs (thin arrows) as larval and pointed abdomen (thick arrow) as pupal characters following haemolymph withdrawal from late V instar larva. Fig 4– Normal V instar larva. Fig. 5– Pharate adult following haemolymph withdrawal (20-30µl) from pupa. Fig. 6– Butterfly with folded / crumpled wings (both sides) following haemolymph withdrawal from late V instar larva. Fig. 7–Butterfly with normal fore-wing on right and folded wings on left side following 50 µl haemolymph withdrawal from prepupa.
Fig. 8– Normal butterfly.

All scales on photographs represent 1.0 cm.

stage larvae also died if the blood was drawn 4 times in a day. In rest of the experimental groups belonging to early and late V instar larvae and pupae, the repeated withdrawals yielded either larval-pupal intermediates (L PIs) (cf Figs 3,4) or pharate adults (Fig 5) or caused deformity in newly emerged adults (Figs. 6– 8) *viz.* short body and wing lengths and folded/crumpled fore-and hindwings It was observed that even a single withdrawal of 100µl blood produced LPIs in III group of late V instar larvae. It could also be derived from Table 3 that if the interval between two withdrawals was extended for 48 hrs, all emerging adults were normal having flight-ability though they were smaller in size with respect to their controls. The side of haemolymph withdrawal did not elicit any effect on the length of wings (not shown in the table).

The measurements of the body length and wing-expanse showed that the control adults had a body 2.5±0.1 cm long and the wing-expanse of 3 9±0 1 cm and 2.8±0.09 cm for fore-and hindwings, respectively (Table 3) But the butterflies emerging after repeated haemolymph removals had a body length of 2.1 ± 0.01 cm and forewing-expanse of 2.9±0.1 cm only.

Discussion

It was Jones[1] who in 1967 examined the effect of repeated haemolymph withdrawals on DHC and found that up to five withdrawals from both the fed and unfed V instar *R prolixus* could not grossly affect the subsequent changes in the haemocyte picture. He however, did not investigate its effect on 'THC. In present investigation, the repeated withdrawals of blood have not only caused considerable variation in DHC but also showed a marked reduction in THC. A similar observation was made by Pandey *et al.*[2] in *P demoleus* It is assumed that this reduction / variation in haemocyte counts (both total and differential) may be brought about either i) by the reduction in blood volume as a result of its repeated removal or ii) by release of haemocytes out of the body through oozing blood after an injury or iii) by damage of cells following repeated puncturing of larval integument.

The increase in PRs count in experimental insects is supported by an increase in their mitotic indices (MI) which play an important role in maintaining abundance of circulating haemocytes. A number of similar observations on increased mitotic activity under certain stress conditions (e.g. bleeding, injury etc.) have been made in other insect spp[12]. The high MI in PRs is, therefore, not surprising in view of their nature as stem-cells that give rise to certain other haemocyte types as has been reported earlier.[11-16].

Table 3– Effect of repeated haemolymph withdrawals on imaginal moulting and body /wing length in *D chrysippus* (Values are m SD for 10 insects).

Experimen -tal groups		Body weight range (mg)	Haemolymph withdrawal		Volume of Haemolymph withdrawn (µl) in		Effect on				
			No of times/ day	No. of days	Single with- drawal	Repeated with- drawals	Pupal moulting	Imaginal moulting	Length of adult (cm)	Length of forewing (cm)	Length hindwi (cm)
Early V instar larvae	I	125±25	1	6	25	150	All died as larvae	–	–	–	–
	II	235±15	1	3	25	75	Occurred	Occurred, all adults abnormal, unable to fly	2 1±0.07	2 9±0 1	Folde Crumpl
			1	4	25	100	Occurred	Pharate adults	–	–	–
	III	300±10	1	2	70	140	All died as larvae	–	–	–	–
			1	2 (at 48 hr interval)	50	100	Occurred	Occurred, all adults normal, able to fly	2.2±0.09	3 0±0 08	2 5±0.(

Table 3 Contd..

Table 3 Contd

Late V instar larvae	I	515±15	2	1	100	200	LP1	–	–	–	–
			3	1	70	210	LP1	–	–	–	–
	II	575±10	1	1	80	80	Occurred	Occurred, all adults abnormal, unable to fly	2 2±0 1	Folded/ Crumpled	Folded/ Crumpled
			4	1	80	320	All died as larvae	–	–	–	–
	III	685±5	1	1	100	100	LP1	–	–	–	–
			1	4	100	400	LP1	–	–	–	–
	IV	580±10 (Prepupa)	1	1	50	50	Occurred	Occurred, all adults with normal wings on right and folded wings on left side	2 3±0 01	3 0±0 06	Folded/ Crumpled
Control		All weight range	Matched mock-punctured unbled larvae				Occurred	Occurred, all adults normal, able to fly	2.5±0 1	3.9±0 1	2 8±0 05

Table 3 Contd ..

Table 3 Contd...

			1	3	10	30	–	Pharate adults	–	–	–
Pupae	I	440±25	1	1	10	10	–	Occurred, all adults abnormal, unable to fly	2 2±0 06	Folded/ Crumpled	Folded/ Crumpled
	II	485±15	2	1	10	20	–	Pharate adults	–	–	–
			3	1	10	30	–	Pharate adults	–	–	–
Control		All weight range	Matched mock-punctured unbled pupae				–	Occurred, all adults normal, able to fly	2 5±0 1	3 9±0 1	2 8±0 05

The role of PLs in injury repairing is well known[12]. Though, Wigglesworth[17,18] believed that haemocytes were not directly involved in wound repair in *R. prolixus*, it was actually performed by epidermal cells. PLs around the wound divide mitotically, migrate and send out cytoplasmic processes to seal off the wound. Whereas, Lai-Fook[19] in the same insect sp. and Rowley and Ratcliffe[20] in *Galleria mellonella* have shown the direct role of PLs and GRs in wound repair. The reduction in the number of PLs following haemolymph withdrawal, therefore, seems to be due to their involvement in wound healing and thus making them unavailable in circulating haemolymph.

Several workers suggested a phagocytic role to GRs[12,13,21] but investigators like Arnold and Salkeld[22] and Saxena *et al.*[23] reported their conversion into SPs, ADs and coagulocytes (COs). Since the number of GRs remains more or less similar in both the groups of larvae, their phagocytic role seems to be secondary. The SPs are known to contribute to energy metabolism of pupa by synthesising fats from carbohydrates and / or proteins[24]. The increase in the count of SPs following haemolymph removal may, thus, indicate their role in energy production probably because of more energy requirement by the wounded larvae in cellular immunity and tissue repair than the control ones. A decrease in the count of PLs may be correlated with a corresponding increase in the number of OEs in experimental larvae.

Furthermore, it is known that the emergence of adult from the old pupal cuticle and expansion of the wings lying crumpled under the old skin need a definite pressure of the blood[3]. It is, therefore, assumed that any change in the pressure of the body fluid will impede the eclosion process and inflation of the wings. The failure of normal ecdysis accompanied with the occurrence of folded/crumpled wings in emerging adults from experimental larvae and pupae in contrast to normal eclosion and wing-expansion in matched mock-punctured insects are likely supposed to be caused by reduction in the quantity of the body fluid following haemolymph withdrawal and not by integumental injury. As haemocytes have been implicated in insect moulting, it is suggested that the retardation in morphogenetic events (production of LPIs and pharate adults) is brought about by reduction in blood volume and, in turn, by reduction / variation in total and differential counts of the blood cells respectively, probably by bringing physiological and endocrinological changes in insect body.

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Larval history of a freshwater prawn from tarai region of Kumaun Himalaya

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Abstract

Under laboratory conditions, the brooding specimens of *Macrobrachium hendersodayanum* released the larvae after 25 to 35 days-at a -water temperature of 25 ± 3°C The larval stages consisted of two advanced zoeal forms, a postlarva and a juvenile Feeding started from the stage of postlarva when the same became benthotrophic in habit. The presence of a tail fan with 21-23 processes in the first two larval stages is an interesting characteristic of larval development of this prawn. The larval development is of "complete suppression" or "totally abbreviated" type and compares well with the type III development of Sollaud[1]

(**Keywords :** larval history/freshwater prawns/Kumaun Himalaya)

Introduction

The crustacean larvae have attracted the attention of biologists since olden times but most of the earlier studies were purely academic. Lately, the motive of studying the larval history of prawns has been specifically management-oriented and aimed at large scale production of the seed of desirable species for culture. However, the literature reveals that in contrast to a great deal of work done on the history of penaeid larvae[2-5], the post-hatching developmental histories of the freshwater prawns are less known[6,7]. *Macrobrachium hendersodayanum* is an exploitable minor prawn species which is widely distributed in the tarai waters of Kumaun Himalaya. Being an important food resource for the poor local folk, it is fished out extensively and indiscriminately and, therefore, a fast depletion in the natural population of this prawn is being noticed. This situation warrants the culture of *M. hendersodayanum* on commercial scale by introducing their seeds into tarai waters of Kumaun region. Therefore, attempts were successfully made to raise the seeds of *M. hendersodayanum* under laboratory conditions. The present paper describes the characteristics of the larvae and juveniles of this prawn from seed identification point of view.

Materials and Methods

Freshly oviposited, berried specimens of *M hendersodayanum,* collected from river Parveen in village Jasari, tehsil Khatima (alt. 207.60 m a.s. l.; 29° 0' N Lat. & 79° 45' E. Long.) in the tarai area of district Udham Singh Nagar in Uttaranchal State were carefully transferred to the laboratory in live, uninjured and unstressed condition. 20 such animals were maintained in dechlorinated tap water in four glass aquaria of 50 l capacity (5 animals per aquarium), having continuous aeration and thermostatically regulated water temperature (25 ± 3°C) at which maximum hatching occurs (about 90 % in this species). The brooding females were provided with algae and fish meat as food which they consumed *ad. libitum.* For the morphological study of the larval stages, eggs were hatched in aquaria and then individual larvae were transferred to separate submerged beakers, covered with cloth mesh. After every moult, a few larvae belonging to each developmental stage, and the newly formed juveniles were fixed in 5% formalin for their microscopic study. Tender green algae and artificial feed were used as food for the moulting larvae and juveniles during their rearing in laboratory.

Observations

Under laboratory conditions, the duration between brooding and hatching at 25 ± 3°C was 25 to 35 days. A pilot experiment, conducted to know the optimum temperature for hatching under laboratory condition, showed that at temperatures below 20°C, hatching does not occur and the brood gradually disintegrates. It was also not possible to hatch those eggs which were separated from the brood. The larval stages consisted of two advanced zoeal forms, here called larva I and larva II, a postlarva and a juvenile stage. The average period of moulting at the above mentioned temperature was 4 days for the larva I & II stages and 6 days for the postlarval stage.

It took about 2-3 days for all the eggs of a berried female to hatch. Soon after hatching, the stage I larvae tended to cling to some part of their mother's body for 1-2 days before they became independent. When kept in beakers, the larvae I & II stuck to the wall of beaker just below its brim. The postlarvae, however, became benthotrophic and crawled on the bottom of the beaker. Active feeding started from this stage only.

Larva I

On hatching, the larva of stage I is about 6.0 ± 0.08 mm in total length. The rostrum is well formed, almost like that of adult and has 5 teeth dorsally, including the

post orbital one, and three teeth on the ventral side It over reaches the antenular peduncle slightly. The rostrum has fine setae in between the teeth. The carapace is also quite similar to that of adult but is completely smooth, without post antennal and hepatic spines. The eyes are sessile and fused with the orbital notch. The ocellated area is small. The abdomen is adult like and consists of 6 segments. Yellowish-brown chromatophores are distributed all over the body.

The antennule has a three segmented peduncle and two small feelers. The stylocerite and statocyst are indistinguishable but the anterolateral spine is apparently formed. The peduncle has a few plumose setae. The inner flagellum 5 segmented. The basal segments of the two branches of outer flagellum fused. All the three flagella tipped with a few spinules. Antenna with almost adult characters. It has a 3 segmented peduncle and well developed endo- and exopodites. The flagellum has upto 15 segments. The scale has setae along its inner margin and its outer spine is quite delicate though prominent. The mandible is without palp. It is clearly differentiated into incisor and molar processes but the teeth are not visible. The maxilla shows the formation of both the gnathobases but they are devoid of setae. Palp only as a protuberance and undivided. II maxilla is much better developed. Scaphognathite is a fan shaped expanded structure and has about 20 plumose setae along its margin. Endopodite narrow and endopodite as well as the gnathobases asetose.

All the three pairs of maxillipeds are apparently formed though they are structurally incomplete. A few plumose natatory setae are present at the tips of their exopods. The coxa and basis of I maxilliped not yet produced into gnathobases. The endopod is small, unsegmented and provided with three setae at the tip. The basal part of exopod with a well marked and expanded flap. The epipodite not distinguishable uptil now. The endopodites of II & III maxillipeds well developed and clearly segmented into 5, bearing a few non-plumose setae along their lengths. The pereiopods, all the 5 pairs, besides their coxa and basis divisions, have segments as in adults but are completely smooth. Exopods not traceable. I and II pereiopods have become chelate.

The abdomen six segmented, the sixth one being elongated and tapering posteriorly like that of the adult and fastened to telson. Except the uropod, which has not developed yet, all the remaining 5 pairs of pleopods have great similarity to those of the adult. The endopod of the I pleopod is rudimentary. The appendix interna yet to be formed. The margins of endopod and exopod in all the five pairs of pleopods beset with minute setae but 3-4 setae on their tips are enlarged and plumose. The telson and uropods (uropods being visible through cuticle as uropod buds only) not differentiated

and represented as a tailfan which broadens posteriorly and is semicircular in shape. The posterolateral margin of tailfan beset with 21-23 processes.

Larva II

The total length is 6.075 ± 0.12 mm. The rostrum and its relationship with antennular peduncle is the same as before. There is also no change in carapace and the distribution of chromatophores. Eyes stalked but free from orbital notch.

In antennule, the statocystic area has been demarcated and the stylocerite developed. The anterolateral spine becomes more prominent. All the three flagella get longer and there is slight increase in the number of segments also in the inner as well as the longer branch of the outer flagellum. No remarkable change occurs in the antenna except an increased length of the flagellum which now contains more than 30 segments. In mandible, the molar and incisor processes develop one tooth each in the middle as a small conical projection. The gnathobase of the I maxilla, formed by basis, has three spinules now and the palp becomes conical. A few spinules develop on the gnathobases of the II maxilla also.

Not much change occurs in the maxillipeds except that the epipodite appears on the I maxilliped and the endopodite of the II maxilliped gets curved towards the inner side and develops plumose setae at the tip. The pereiopods are as in the previous stage but they become sparsely setose.

The second larval stage does not show any further modification in the abdomen or in pleopods and so is the case with tailfan and its processes. However, the uropod buds appear to have been transversely divided at this stage.

Post larva

The postlarva attains an average total length of 6.79 ± 0.38 mm. The eyes become slightly more enlarged. In rostrum, the number of teeth increases to seven on the dorsal side and four on the ventral side.

The antennule shows the development of three aesthetascs at the tip of the smaller branch of the outer flagellum. The antennule flagellum with about 45 segments now. The incisor process of the mandible deeply notched to form two teeth and the mandibular palp makes its appearance as a bud on the outer side of the incisor process. In I maxilla, thickened spinules are present on both the gnathobases. A clearcut bifurcation takes place in the palp and the terminal half, thus formed, is acutely pointed. No change occurs in II maxilla in comparison to the earlier stage.

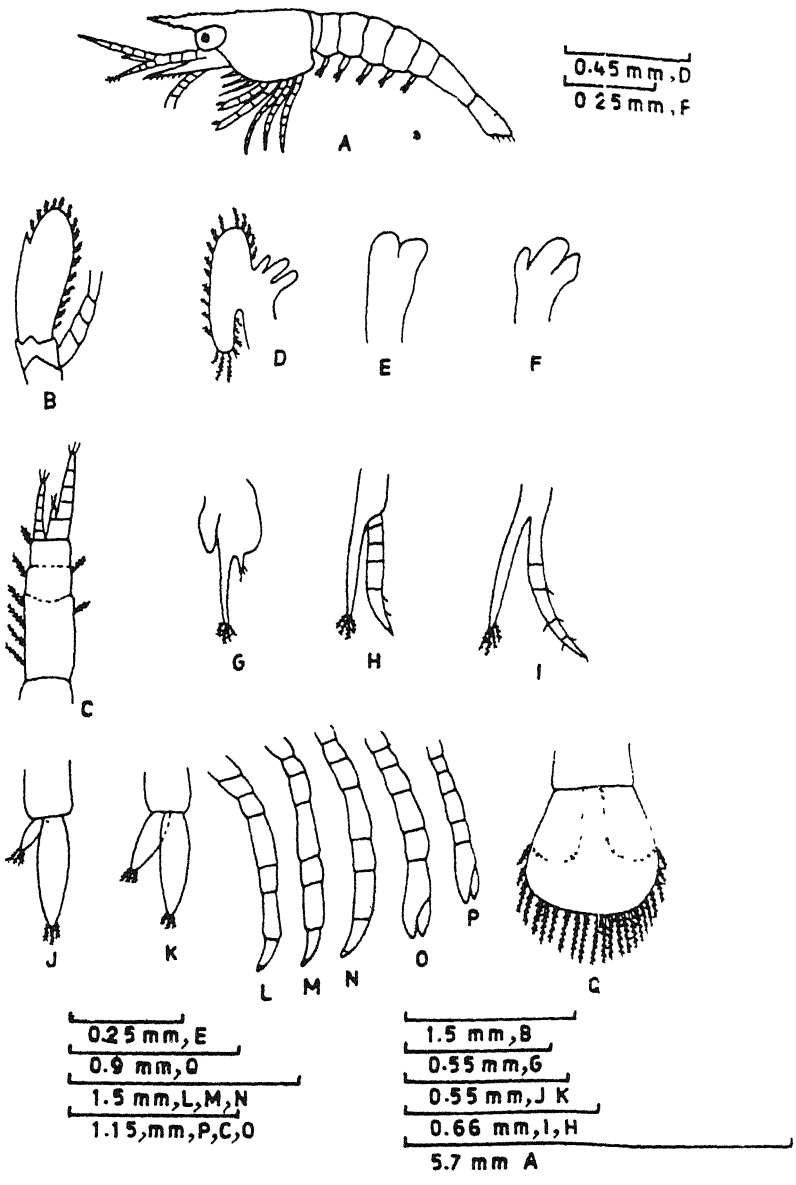

Fig. 1– Morphological characteristics of larva I of *M hendersodayanum*

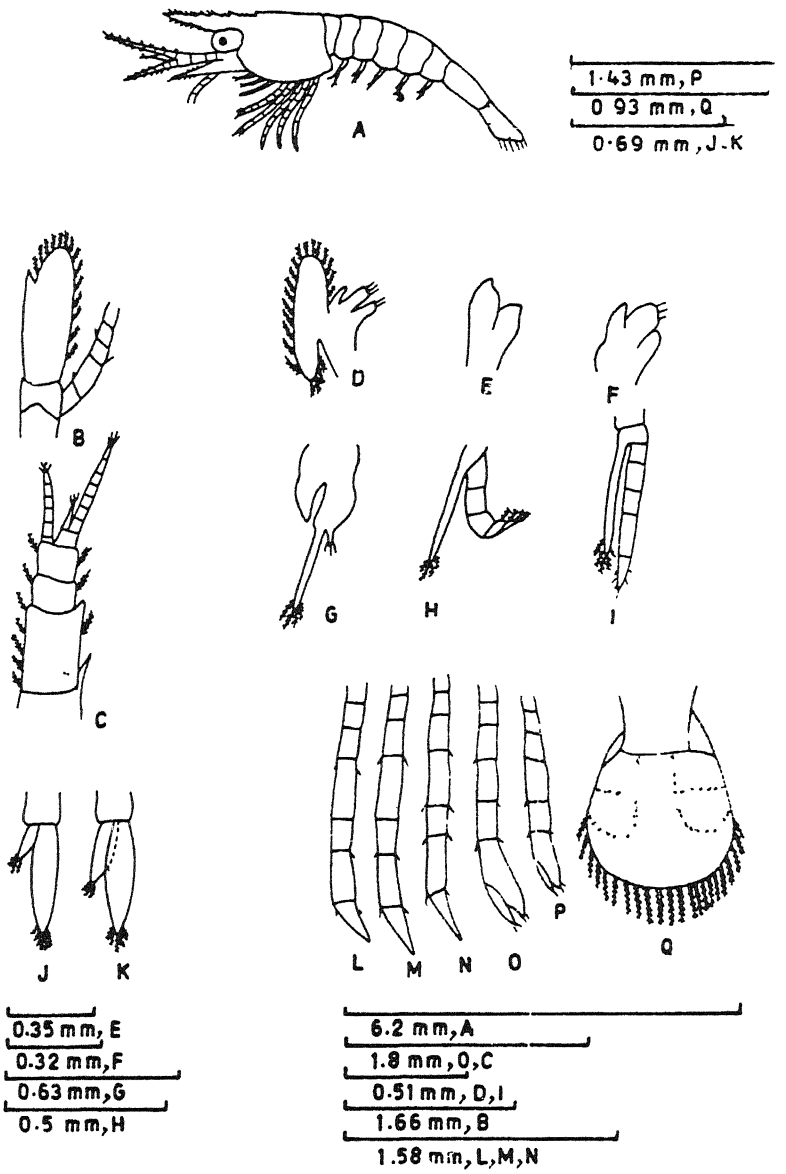

Fig. 2– Morphological characteristics of larva II of *M hendersodayanum*

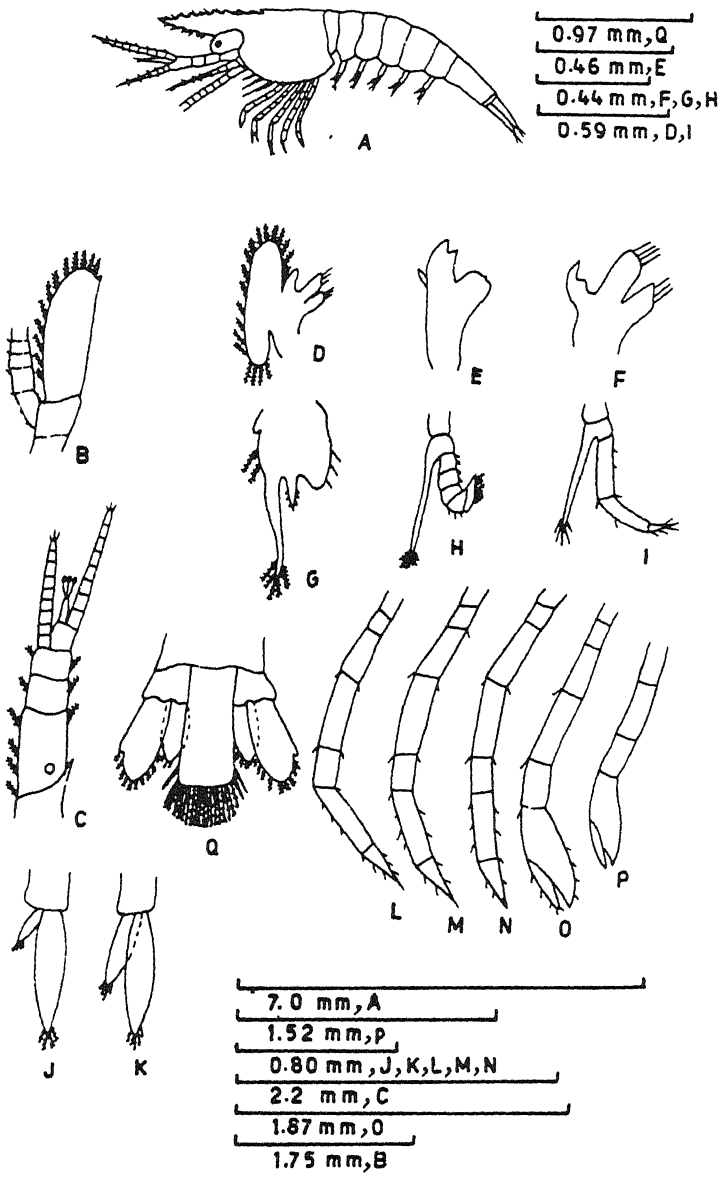

Fig. 3– Morphological characteristics of the postlarva of *M hendersodayanum*

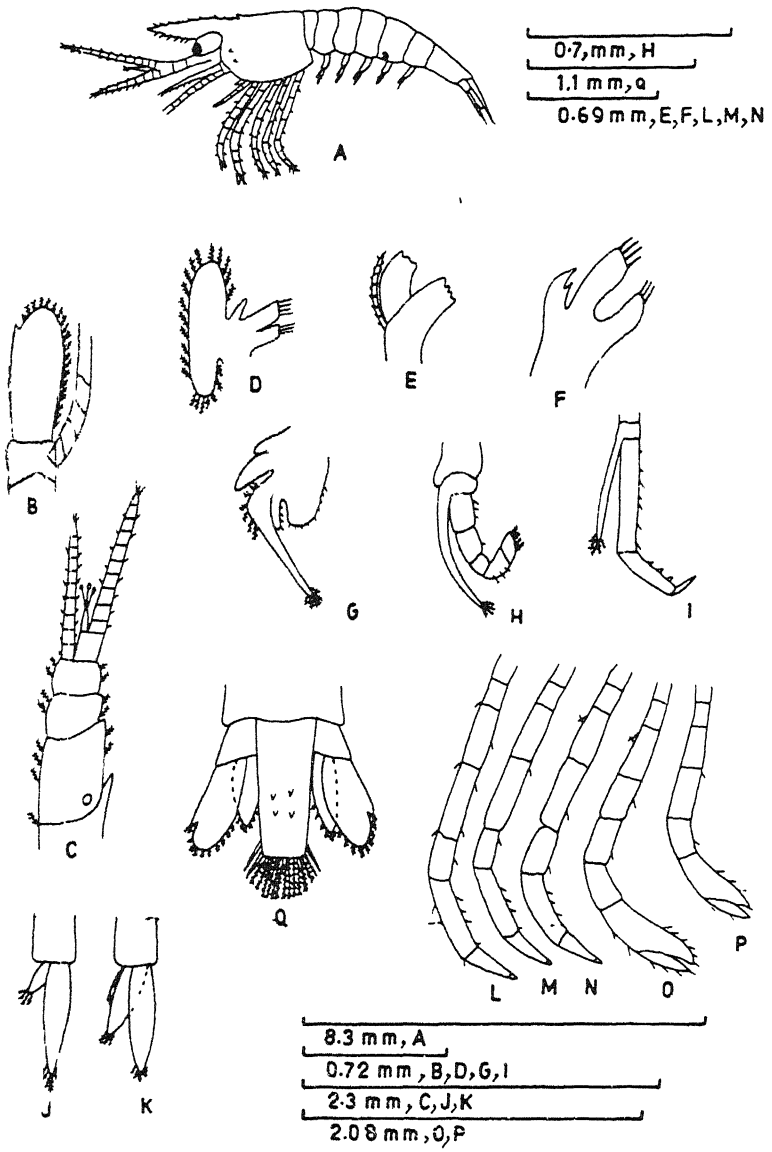

Fig 4– Morphological characteristics of the juvenile of *M hendersodayanum*

In I maxilliped, the coxal endite (gnathobase) smooth but the basal endite develops 2 or 3 setae. No change occurs in the II maxilliped. The endopod of the III maxilliped now left with 3 segments only (showing fusion of ischium & merus on one hand and that of propodus and dactylus on the other). Except for a slight elongation in size and the growth of setae, the pereiopods remain as in the earlier stage.

The pleopods are without any change. However, the tailfan gets clearly differentiated into a pair of uropods and a long and rectangular telson. The telson is slightly broader posteriorly and has a mild notch in the middle, but is still unlike that of the adult. Out of all the telson processes of the earlier stage, the first two pairs get converted into conical spines. The uropods at this stage are functional, and are provided with both the rami which are densely setose. Posteriorly, the uropods reach upto the end of telson.

Juvenile

The first formed juveniles attain an average total length of 7.88 ± 0.43 mm. They are almost like the adults in general appearance. The eyes get still longer and are freely movable within the orbital notch. The ocellated area becomes much larger and bulging. Rostrum same as earlier. The post antennal and hepatic spines, unnoticeable until the earlier stage, are quite apparent in the juvenile stage and occupy their usual positions.

In the antennule, the smaller branch of the outer flagellum adds one more segment to it and is tipped with 3 aesthetascs as in the earlier stage. The longer branch has 11 segments. The inner flagellum has 10 segments. The antennular flagella of the juvenile prawn become setose throughout. The antennal flagellum adds 3-4 segments to it. The mandible exhibits 3 teeth in the incisor and 4 in the molar processes. A well developed and segmented palp is formed on the outer side of incisor process. Spinules are found on both the gnathobases of I and II maxillae but those of the II maxilla get stronger.

The maxillipeds do not present any appreciable change except for an increase in their size and setation. The epipodite of I maxilliped becomes distinctly bilobed and the II maxilliped is provided with a podobranch. The pereiopods are different from the earlier stage in having a greater number of setae only.

Abdomen almost as in the previous stage. An elongated bud like future appendix interna becomes visible on all the pleopods from 2nd to 5th. The telson is narrower than in the previous stage and its posterior margin has become straight. Two pairs of short spines appear posterodorsally on the telson. The arrangement of processes

remains unchanged. The uropods are almost like those of the postlarva in structure and size.

Discussion

Among the freshwater prawns, larval development has been studied in *M lamarrei*[8], *M. malcolmsonii*[9] and *M kistnensis*[6,10] According to Sollaud[1], 3 types of larval developments have been described in caridean shrimps. In type I, also called as the "common" or "typical" type of larval development which is exemplified by *M acanthurus*[11], *M. malcolmsonii*[9], *M carcinus*[12], *M intermedium*[13], *M rosenbergii*[14] and *Palaemon ortmanni*[15], a large number of small eggs, along with an extended life history having several free swimming larval stages are found. In type II, also called as the "abbreviated type" of larval development as exemplified by *M. lamarrei*[8] and *M potiuna*[1], the animals have large-sized but lesser number of eggs and free swimming larval stages. The third type or the "complete suppression" or "totally abbreviated" type which is exemplified by *M. shokitai*[16], is characterized by the occurrence of fewer eggs than in " type II " and the minimum number of larval stages On hatching, the larva is non-free swimming and it almost resembles the adult or the postlarva.

Considering the facts that the number of eggs produced by the present species is relatively low and the larva on hatching is non-free swimming and it greatly resembles the adult, the larval development of *M. hendersodayanum* appears to be similar to the "type III" of Sollaud[1]. The totally abbreviated life cycle in the concerned prawn (in which almost all organs are formed even before hatching) is also exhibited by the presence of a well developed antennule, antenna, II maxilla as well as the pereiopods which are uniramous and without exopods even in the first hatched larva. The first hatched larva of this species first sticks to the body of the mother and then to the wall of the aquarium or beaker and does not feed at this stage. After passing through another larval stage, it is converted into a postlarva which of course, becomes benthotrophic and starts feeding. The first two larval stages of *M. hendersodayanum*, which show only slight modifications in the mouth parts and rostrum, may therefore be considered as the advanced zoeal stages. The postlarva characteristically has a functional biramous uropod. In *M. shokitai*[16], two zoeal and one megalopa stages have been identified before the juvenile stage[16]. However, since the functional biramous uropod in *M. shokitai*[16] is not observed before the juvenile stage, the juvenile of *M. shokitai* may be considered as the postlarva and, therefore, instead of two, *M. shokitai* seems to have three zoeal stages. The presence of a rounded fan-shaped telson with a certain number of marginal processes as also seen in *Palaemonetes sinensis*[17], *M. shokitai*[16], *M. kistnensis*[6] and *Palaemon ortmanni*[15] has been considered to be a unique

feature of *Macrobrachium* larva In this regard, *M. hendersodayanum* is similar to the aforesaid species because the I and II stage larvae of *M hendersodayanum* are provided with a prominent tailfan with 22-26 processes which exactly resembles with the telson described by Shen[17], Shokita[16] and Jalihal *et al.*[6].

Abbreviations used

A . Entire larva, B . Antenna, C : Antennule, D : Maxilla (or II maxilla), E . Mandible, F : Maxillula (or I maxilla), G: I Maxilliped, H : II Maxilliped, I : III Maxilliped, T J : I Pleopod, K : III Pleopod, L : V Pereiopod, M : IV Pereiopod, N : III Pereiopod, O . II Pereiopod, P: I Pereiopod, Q : Tail fan

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Nutritionally important constituents and nutritional value of green mussel *Perna viridis* (L.) from Northwest coast of India

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Abstract

The concentration of soluble carbohydrate, crude protein, organic matter, caloric value, edibility and condition index in *Perna viridis* were ranged from 13 44-14 21, 52 12-58 37, 78 56-81 32 mg $100g^{-1}$, 297 92-333 42 Kcal $100g^{-1}$, 47 52-53 48% and 40 90-47 28 respectively at Mocha and Dwarka Similarly, the total and unsaponifiable carotenoids were 35 48 and 32 01 % respectively more Dwarka than Mocha The crude fat was varied from 8 28 at Dwarka and 8 87 mg $100g^{-1}$ at Mocha The concentration of inorganic constituents such as calcium, magnesium, sodium and iodine were comparatively more at Dwarka than Mocha However, reverse trend was observed for total, soluble and insoluble ash and potassium The concentration of calcium (2125mg $100g^{-1}$) was especially very high at Dwarka The biomagnification of iron, manganese, cobalt, cadmium, zinc and nickel were maximum at Dwarka while copper and lead were maximum at Mocha Mercury was below detection limit at both the places However, the concentration of toxic heavy metals at Mocha and Dwarka, were many magnitude less than maximum permissible limit prescribed for human consumption The present study indicates that the nutritionally important constituent/parameters in *P viridis* at Mocha and Dwarka were in the range which makes it suitable for utilization as food

(**Keywords** food/molluscs/*Perna viridis*/nutrition/micronutrients)

Introduction

Perna viridis (earlier named *Mytilus viridis)* was reported for the first time on Gujarat coast[1]. However, this had widespread occurrence in India[2]. The heavy metal content of this species has been reported from different parts of India[3-7]. Similar studies with *Mytilus* and other bivalves has been reported from different parts of the world[8-12]. Very little work has been done on the biochemical composition of *P. viridis* to identify its nutritional potential for human consumption[13-15]. The biochemical composition of *P. viridis* from Gujarat coast has not been studied Gujarat is a first

largest industrialized state of India and its coast is considerably polluted due to industrialization of this area[16]. Since it is used as food, therefore it is desirable to know its biochemical composition and nutritional quality to evaluate its food potential.

Materials and Methods

The green mussel *Perna viridis* (L.) were collected from the intertidal belt of Mocha (Lat. 21° 20'N, Long. 69° 53'E) and Dwarka (Lat. 22° 25'N, Long. 68° 04'E), Saurashtra coast of Gujarat during September. They were collected from the region at 0.21m above the zero of the *chart datum.* Soon after collection the epiphytic flora and fauna were thoroughly removed and washed with clean seawater. Fifty mussels from each station were collected randomly. They were preserved in ice cold condition and transported to the laboratory where the morphometric measurements were made by vernier caliper and analytical balance. The mussels having a mean length of 82.72 ± 1.82mm at Mocha and 118.28 ± 1.92 mm at Dwarka were utilized for the present investigation. The valves of the mussels were cracked by hammer and the soft tissues were removed gently by teflon scalpel and weighed individually. The tissues were pooled together and rinsed with deionised water. Further the samples were dried at 55°C till constant weight for the estimation of organic and inorganic constituents as well as heavy metal concentrations. Later on they were ground to fine powder. The 100μ mesh sized powder was used for the above said estimations.

The soluble carbohydrate and crude fat were determined by the method described by Dubois *et al.*[17] and Folch *et al*[18]. The crude protein was determined by a micro-Kjeldahl method[18]. The total, soluble and insoluble ash, calcium, magnesium, sodium and potassium were estimated by the method described by Humphries[20]. The concentration of iodine was determined by the method of McLachlan[21]. Heavy metals were analyzed spectrophotometrically as per the method described by Tewari *et al*[22].

The caloric value was calculated by multiplying crude protein, soluble carbohydrate and crude fat values (in grams) by factor of 4.0,4.0 and 9.0 respectively[23]. The organic matter was determined by the method described by Wakeel and Riley[24]. The edibility was calculated as the amount of water content in relation to the total weight of mussel meat[14]. The Condition Index (CI) has also been calculated using following equation described by Walne[25].

$$CI = \frac{\textit{Mean dry meat weight (g)}}{\textit{Mean dry shell weight (g)}} \times 100$$

The total and unsaponifiable carotenoids were determined by the method of Karnaukhov *et al.*[26] The geographical position of sampling station were measured by Global Positioning System, Model JLR-4400.

Results and Discussion

The concentration of organic constituents, caloric value, edibility and condition index of *P. viridis* at Mocha and Dwarka is presented in Table 1. The soluble carbohydrate, crude protein and organic matter were comparatively more at Dwarka than Mocha. These parameters were 5.73,11.99 and 3.51% more at Dwarka as compared to Mocha. Similarly, total and unsaponifiable carotenoids were 35.48 and 32.01% respectively more at Dwarka than Mocha. However, the reverse trend was observed for crude fat. The crude fat was 6.65% less at Dwarka than Mocha. Similarly the caloric value, edibility and condition index were also correspondingly higher in *P. viridis* at Dwarka as compared to Mocha. These parameters were 11.99, 12.54 and 15.59% more respectively at Dwarka than Mocha.

Table 1– Organic constituents, caloric value, edibility and condition index of *P viridis* at Mocha and Dwarka (g $100g^{-1}$ dry wt)*

Constituent	Mocha	Dwarka
Soluble carbohydrate	13 44	14 21
Crude protein	52 12	58.37
Crude fat	8 87	8 28
Total carotenoid (mg g^{-1})	3 60	5 58
Unsaponifiable carotenoid (mg g^{-1})	1.72	2 53
Organic matter	78 56	81 32
Caloric value (Kcal $100g^{-1}$)	297.92	333.42
Edibility (%)	47 52	53 48
Condition index	40.90	47.28

*Average of 5 replicates

The inorganic constituents of *P. viridis* from Mocha and Dwarka is depicted in Table 2. The concentrations of total, soluble, insoluble ash and potassium were less in Dwarka samples as compared to Mocha samples It was 13.04, 24.96, 7.72 and 19.47% less respectively at Dwarka than Mocha. However, the reverse trend was observed for calcium, magnesium, sodium and iodine. These constituents were 82.25, 14.99, 17.26 and 19.28% respectively more at Dwarka as compared to Mocha.

Table 2– Inorganic constituents of *P viridis* at Mocha and Dwarka (mg $100g^{-1}$ dry wt)*

Constituent	Mocha	Dwarka
Total ash	21481	18679
Soluble ash	6610	4960
Insoluble ash	14869	13720
Calcium	1166	2125
Magnesium	539 2	620 0
Sodium	1 97	2 31
Potassium	0 606	0 118
Iodine	61 337	73 160

*Average of 5 replicates

The concentration of different heavy metals in *P. viridis* at Mocha and Dwarka is given in Table 3. The biomagnification of iron, manganese, cobalt, cadmium, zinc and nickel were more in Dwarka sample as compared to Mocha sample. It was 163.71, 1257.14, 157.14, 59.93 and 5.45% more at Dwarka. However, the reverse trend was observed for copper and lead. They were 8.36 and 42.86% less at Dwarka as compared to Mocha. The mercury was not detected in anyone of the sample. The concentration of poisonous metals in *P. viridis* were quite often many magnitude less than the maximum permissible limit prescribed for food products (cf. Table 3)[30].

In general Dwarka mussels has higher concentration of nutritionally important constituents than Mocha animals. The concentration of calcium in Dwarka was especially very high. The concentrations of toxic heavy metals in *P. viridis* at Mocha

and Dwarka were many magnitude less than maximum permissible limit prescribed for human consumption. All the results reported here were highly significant ($P < 0.01$, $r = 1$). The above said results indicate that nutritionally important constituents/parameters of *P. viridis* at Mocha and Dwarka were in the range which makes it suitable for utilization as human food.

Table 3– Heavy metal concentration of *P. viridis* at Mocha and Dwarka (mg $100g^{-1}$ dry wt.)*

Heavy metal	Mocha	Dwarka	Maximum permissible limit* (ppm)
Iron	10.36	27.32	Not specified
Manganese	0.112	1.520	Not specified
Cobalt	0.720	0.912	Not specified
Cadmium	0.007	0.018	1.5
Copper	0.383	0.351	30.0
Lead	0.721	0.412	2.5
Zinc	4.283	6.850	50.0
Nickel	0.110	0.116	Not specified
Mercury	BDL	BDL	1.0

* Average of 5 replicates

BDL- below detection limit

Edible bivalves especially mussels being filter feeders exhibit high conversion efficiency and consequently have high food value and elevated contents of the major biochemical constituents of body tissue in terms of quality and quantity. The information on biochemical composition is essential as it reflects directly on the nutritive value, thereby enabling to suggest an ideal time for subsequent harvest of the crop[15]. In the present study the concentration of soluble carbohydrate, crude protein and crude fat is in range of 13.44-14.21, 52.12-58.37 and 8.28-8.87% respectively. These ranges in *P. viridis (M. viridis)* are well within the range (5.00-25.00, 47.00-65.00 and 2.50-16.20% respectively) reported from the different coast of India[13-15] and United Kingdom[8]. The caloric value in the present study ranged from 297.72-333.42 Kcal $100g^{-1}$ which is considerably less than (300-1560 Kcal $100g^{-1}$) those reported from Goa[13-15]. However, the variation of edibility (47.52-53.48%) and condition index

(40.90-47.28) in the *P. viridis* from Saurashtra coast were quite comparable to those reported from Goa (15.00-55.25% and 5.00-25.00 respectively)[14].

The per cent composition of total ash in the present study is considerably less (1.87-2.15%) as compared to those reported from Goa (5.0-21.5%)[14-15]. However, the cellular concentration of calcium and magnesium in *P. viridis* from Saurashtra coast (1166-2125 and 539.2-620.0 mg $100g^{-1}$ respectively) were many magnitude more than those reported (112.4-195.0 mg $100g^{-1}$ respectively) from Goa[27]. Therefore the *P. viridis* from Saurashtra coast is having better inorganic nutrient composition than those reported from Goa.

The concentration of iron (10.36-27.32), manganese (0.112-1.520), cadmium (0.007-0.018), copper (0.351-0.383), zinc (4.283-6.850 mg 100g-1) and mercury (below detection limit) in *P. viridis* from Saurashtra coast were much less as compared to *P. viridis/M. viridis* reported from other parts of India and different parts of world (54.93-209.09, 3.186-7.156, 0.105-0.963, 0.860-6.578, 0.590-10.088 and BDL-0.0073 mg $100g^{-1}$ respectively)[3,4,6,27-29]. However, the concentration of cobalt (0.720-0.912) and lead (0.412-0.721 mg $100g^{-1}$) were quite comparable to those reported from other parts of India (0.105-0.737 and 0.154-0.985 mg $100g^{-1}$ respectively)[4,6,26] and from other parts of the world (0.0029-0.870 and 0.19-1.27 mg $100g^{-1}$ respectively[29]. The concentration of total and unsaponifiable carotenoids in *P. viridis* from Cochin were significantly less (0.31 to 2.20 and 0.11 to 1.75 mg g^{-1}) as compared to the present study[31]. The carotenoid has nutritional value as certain carotenoids like α and β carotene and cryptoxanthin are converted to provitamin A in the liver of many animals[32]. The environmental stress caused due to heavy metal and oil pollution, may also increase in carotenoid concentration in molluscs, this effect is due to the carotenoids take part in oxygen metabolism of animal cells providing an intracellular reserve (accumulator) of oxygen (or its electron-acceptor equivalent), which makes it more resistant to heavy metal pollution or hypoxic conditions[26,31].

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Traditional folk medicines of the *Shepoumaramth* Nagas of Senapati district in Manipur

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Abstract

Senapati district of Manipur has cool and salubrious climate that favour the growth of diversity of medicinal plants About seventy two medicinal plant being used by *Shephoumaramth* Nagas as folk medicines have been identified and documented along with their curative properties for the treatment of numerous human diseases. Use of medicinal plants and cultural ethics are closely linked among the *Shephoumaramth* Nagas and they possessed high conservative and descended through generations. They keep outmost secrecy of the curative properties of the plants in the form of oral and traditional ethics which help in the conservative management of rare and endangered medicinal plant species of the district. Most of the medicinal plants are herbs followed by shrubs and a few trees available at different seasons of the year.

(Keywords : Nagas/folk medicine/floristic diversity)

Introduction

Situated in between 24° 37″ and 25° 25″ North latitudes and 93° 45″ and 94° 29″ East longitudes, Senapati Senapati is one of the 9 districts in Manipur covering 3271 square km out of the total 22,356 Sq. Km. geographical area of Manipur. Physiographically, the district is more or less hilly tract. The Laimatal range passes through the middle part of the district in north-south alignment forming slopes towards south-west, west and south-east. Out of the total forest area of 2259 Sq. Km., the dense forest area is only 730 Sq. Km. accounting 22.3% of the total area. The rest 1529 Sq. Km. (46.7%) is the open or degraded forest which called for immediate attention. The scrub forest cover 347 Sq. Km (i.e. 10.6%).

The present total population of *Shepoumaramth* Nagas is about one lakh fifty thousands that include Mao, Maram, Poumei and Thangal Tribes. Since time immemorial, local people of this region particularly the Nagas use a large number of

medicinal plants to cure or prevent myraid of diseases from the jaw of death. These medicinal plants include herbs, shrubs and trees. Their methods of preparation includes extraction or squeezing out of leaves juice, stem, fruits, roots, etc. or boiled and the soup or whole plant is taken, rarely mixing with other ingredients. Limited knowledge about the nature of the disease coupled without undergoing any experimental study are the main handicaps to improve their methods of treating the diseases.

The aim of the present study was to document the untapped traditional knowledge of medicinal plants, to sensitize and create awareness among the local people about the importance of medicinal floral diversity in *Shepoumaramth* Naga areas in today's patent regime and to develop suitable strategy to document the traditional knowledge on plant resources. Secondly to encourage the conservational and rational utilisation of medicinal plant resources of *Shepoumaramth* Nagas areas.

Materials and Methods

A total of about hundred ethnobotanical surveys were conducted in *Shepoumaramth* Naga areas. Medicinal plants used by different communities/tribes of the area were identified. Some of the medicinal plants which cannot be identified in the field were collected and standard herbarium was made and taken to Botanical Survey of India (BSI), Shillong, and got identified. Further, most of the medicinal plants used by the *Shepoumaramth* Nagas got identified in local dialect with the help of local herbal practitioners and some village elders known as *Oprokopfumei* having good knowledge about local flora. The information on traditional folk medicine practiced by *Shepoumaramth* Nagas were collected by visiting randomly in selected villages of *Shepoumaramth* Nagas. A pre-designated format and standard questioners were prepared and information's were extracted from the village elders, herbal medicinal practitioners and professional forest user groups. Series of interviews and group discussions were conducted with the villages elders including women. Data on local name, medicinal value, plant parts used and mode of preparation from the medicinal plant parts were collected, recorded and tabulated.

Results

During the ethnobotanical field survey of one year as many as 72 medicinal plants including rare species like *Taxus baccata* and *Panax pseudoginsen* were found to be used by the ethnic group *Shepoumaramth* Nagas. These medicinal plants are used for the treatment of as many as more than 50 human diseases including the dreaded

cancer, diabetes, etc. Different plants parts such as leaves, stem, root, flower, fruit, bark, rhizome etc. are used for the treatment of human diseases. Most of the medicinal plants are found during the specific period of the year. However, there are group of medicinal plants falling under the categories of perennial can be used throughout the year (Table 1).

Table 1– Medicinal Plants used by the *Shepoumaramth* Nagas

Local Names		Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of .	Availability Season/ Periodicity
1	Shyramakiipro (Mao)	*Hemphragma heterophyllum* Wall (Scrophular iac-eae)	Herb	Whole plant	The plant is washed, boiled and the soup is drink	Fever in cattle, dog and human	May to September
2.	Oramakipro (Mao)	*Sarcopyranis nepalensis* Linn (Melastomataceae)	Herb	Whole plant	The plant is washed, boiled and the soup is drink	Malarial fever	May to Nov.
3	Eveakorie (Mao)	*Thalictrum foliolosum DC (Ranunculaceae)*	Herb	Root	The roots are washed, crushed and is then taken in raw or boiled and the soup is drink	Fever, stomach ache, hypertension, dysentery, emetic, headache, better digestion and abdominal flatulence.	Perennial
4.	Othukoshii (Mao)	*Ranunculus sceleratus* Linn. (Ranunculaceae)	Herb	Root and leaves	The leaves or roots are washed, crushed and wound over with bandage on the infected part of the body.	Boils, snake bite	May to Nov

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
5 Letikorei (Mao) Creeper	*Hydrocotyl sibthorpioides* Lam. (Umbelliferae; Apiaceae)	Herb	Whole plant	The leaves are washed, the juice is squeezed out and applied	Cut and injury	April to November
6 Changhobou (Mao)	*Adhatoda vesica* Nees. (Acanthaceae)	Shrub	Leaves and flowers	The leaves are washed, boiled and the soup is drink.	Body ache, dry cough, fever, gastric trouble and high blood pressure	Flower-Jan. to Feb
7 Koiralabou /Kilashibou (Mao)	*Physalus peruviane* Linn. Var *edulis* (Solanaceae)	Herb	Leaves	The leaves are washed and taken in raw. Also the leaves are warmed from the fire and massage the body.	Stomach ache, dysentery and body ache.	May to Oct
8. Ozeanabi (Mao)	*Oxalis corniculata* Linn (Oxalidaceae)	Herb	Leaves	The leaves are folded in banana leaf and warmed in the ash of the fire The juice is then squeezed out and given for	Diarrhoea, dysentery, lips and mouth infections.	April to September
9. Emosiibou (Mao)	*Rhus javanica* Linn. (Anacardiaceae)	Tree	Fruits Leaves and flowers	The fruits are boiled with sugar and the soup is then taken orally.	Fever, dysentery, emetic due to intake of contaminated oily food.	Fruit-Oct to Dec

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
10 Napeou (Mao)	*Ocimun sanctum* Linn (Labiatae, Lamiaceae)	Herb	Leaves and inflores cences	The leaves and inflorescences are cooked along with rice or in some cases taken as raw.	Fever, headache, high blood pressure, tonsil and gastric trouble	June to September
11. Chivii (Mao)	*Spilanthes paniculata* DC (Asteraceae, Compositae)	Herb	Leaves and tender stem.	a) The plants are washed, boiled and is taken as medicinal foodstuff. b) The raw leaves taken along with cooked rice and egg yolk is prescribed for	a) Diarrhoea, high blood pressure, abdominal flatulence, etc. b) jaundice	May to September
12. Ohukoshii (Mao)	*Solanum nigrum* Linn. (Solanaceae)	Herb	Whole plant.	The leaves are washed, boiled and the soup is drink.	Kidney disorder, pancreas trouble. The crushed fruits mixed with cooked rice are giving to hen for treatment of fever.	May to September

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
13 Heniatopro and Likhodaphroshi (Mao)	*Geranium nepalense* Sw (Geraniaceae) *Fragaria nigherensis* Schl (Rosaceae)	Herbs	Whole plant	Both the plants are taken in equal amount, washed, boiled and soup is then drink	Stone case in urinary bladder, body swelling or oedema, dysuria/ stranguary The boiled leaves extracts of Likhodaphroshi is also giving to diabetic patients	May to September April to October
14. Mothibou/Ngoathe (Mao)	*Zanthoxylum rhetsa* DC. (Rutaceae)	Tree	Leaf and fruit	The leaves juice is squeezed out and applied to the nose The raw fruits are taken against	Sinus and abdominal flatulence	Fruit-Aug to Sept
15. Ojiipan (Mao)	*Potentilla anserine* Linn (Rosaceae)	Herb	Root	The roots are washed, chewed and the juice is swallowed.	Diarrhoea and Stomach ache	April to October
16. Kohrimalepro (Mao)	*Cajanus cajan* (Linn.)Millsp (Fabaceae, Papilionaceae)	Under shrub	Leaf	The leaves paste is applied for	Boils and swelling cheeks	May to September
17. Kataimai kangaiti (Maram)	*Melothrea maderospatane* (Linn) Cag. (Cucurbitaceae)	Herb	Fruit	The fruit are washed, boiled and the soup is taken orally	Jaundice	July to September

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of.	Availability Season/ Periodicity
18 Trangtrangpa (Maram)	*Verbena officinalis* Linn (Verbenaceae)	Herb	Leaves	The fruit are washed, boiled and the soup is taken orally	Body swelling/ oedema.	April to October
19. Adam Evepa (Maram)	*Catharanthus roseus* (Linn.)Don *(Apocynaceae)*	Herb	Leaves	The leaves are washed, boiled and the soup is drink	Recurrent fever	Perennial
20. Tamsii (Maram)	*Cynodon dactylon* Pers (Gramineae; Poaceae)	Herb	Leaves and young stems	The leaves and tender stems are washed, crushed and is then apply for	Cut/injury.	April to September
21. Napoupro (Mao)	*Chenopodium ambrosiodes* Linn (Chenopodiaceae)	Herb	Leaves and young stem	The leaves and tender stems are warmed from the fire and massage to the body. The leaves extract is also applied to	T B. patients and cut/injury	April to October
22. Veisii (Poumei)	*Cedrella serrata.* Royle (Meliaceae)	Tree	Leaves, roots	a) Leaves are boiled by adding salt. Metta tel oil is initially applied to the dislo-cation/broken bone. The leaves are then placed over the	a) Dislocation, cut and broken bone b) Hypertension and abdominal flatulence.	March to Sept. Perennial

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of .	Availability Season/ Periodicity
				injury part and wounded with bandage b) The roots are washed, crushed and boiled. The soup is then drink.		
23. Orashikhokha (Mao)	*Solanum kurzii* Linn (Solanaceae)	Under shrub	Fruits	The fruits are taken in raw. Also the fruits are cooked with rice and then taken.	High blood pressure, fever, Headache, etc	July to September
24. Pfidigai (Maram)	*Clerodendron viscosum* Vant. *(Verbenaceae)*	Shrub	Leaves	The leaves are washed, boiled and is then taken as medicinal food stuff.	Hypertension.	July to September
25. Kabi (Maram) Kathemei dokre (Mao)	*Oroxylon indicum* (Linn.) Vent (Bignonaceae)	Tree	Fruit, bark and root	The bark of the stems/root is crushed, boiled and drink or is taken in raw.	Gastric trouble, jaundice, blood pressure, blood purification, stomachic, fever, cancer, etc	Oct. to November
26 Khonghosii (Mao)	*Taxus baccata* Linn. (Taxaceae)	Tree	Whole	The leaves decoction are given as a remedy against	Asthma, bronchitis, hiccough, d epilepsy and for indigestion	Whole year

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
					The plant is used as fish poison It is also used for treatment of blood cancer/ Leukaemia	
27. Ekhrou (Mao)	*Pilea umbrosa Wedd* (Urticaceae)	Herb	Leaves	The leaves are warm from the fire and is then pasted in the injury part of the body.	Prevent from further infection caused by germ/bacteria /certain other pathogens.	March to Sept
28 Eshou (Mao)	*Impatiens balsamina* Linn (Balsaminaceae)	Herb	Leaves/ young stem/ branches	The leaves and tender stem/branches are boiled The soup is then drink	Diarrhoea, dysentery and gastric disorder	April to September
29. Raisii (Paomei)	*Betula alnoides* Buch-Ham ex D. Don (Betulaceae)	Tree	Bark of the stem	The bark of the root or stem is crushed and boiled The soup is then drink	Diarrhoea, dysentery and stomachic.	Perennial
30 Chissii (Poumei)	*Quercus serrata* Thumb. (Fagaceae)	Tree	Stem/ branches	The stem/branches are cut and the juice released from cut is collected in the	Acute anal infection The juice without mixing water is taken for	Perennial

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of :	Availability Season/ Periodicity
				bottle One tea spoon of the juice is mixed in one liter of water and boiled by adding 4-5 teaspoon of salt It is then applied	dysentery, Diarrhoea and stomach ache.	
31. Rheohii (Maram) Ahriipro (Mao)	*Mimosa pudica* Linn. (Mimosaceae)	Herb	Root	The roots are crushed/ smashed, mixed with water and drink.	Fever The mixture is also used for the treatment of dental caries	Perennial
32. Chethousii (Mao)	*Melia* □*iarrhea*□ Willd.(Meliaceae)	Tree	Leaves and Fruits	The leaves of this plant along with the peach leaves are boiled together and the soup is then drink	Malaria fever. Also the fleshy soft tissue of the fruits is taken in raw for malaria fever and stomach ache.	Fruit-Oct to July
33. Hraikama (Mao)	*Hedychium spicatum* Buch.-Ham. (Zingiberaceae)	Herb	Root.	The rootstock are washed and boiled by adding salt. It is then taking as medicinal food stuff.	Carminative, stomach ache, stimulant, etc.	Perennial

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of ·	Availability Season/ Periodicity
34 Leriovu (Mao)	*Nasturtium indica* Linn. (Brassicaceae)	Shrub	Leaves	The leaves are washed, boiled by adding salt The soup and the boiled leaves are then taken.	Body ache, malarial fever, etc.	May to September
35 Napeou Kateina (Mao)	*Ocimum basalicum* Linn. (Labiatae; Lamiaceae)	Herb	Leaves	The leaves are boiled by adding chilly and other ingredients as medicinal foodstuff	Headache, common fever and high blood pressure	June to October
36 Shipraipraikro (Mao)	*Artemisia vulagaris* Linn. (Asteraceae, Compositae)	Herb	Leaves	The leaves are washed and juice is squeezed out. It is then drink.	Bleeding during dysentery, □iarrhea etc	May to October
37. Chiihroshibou (Mao)	*Emblica officinalis* Gaertn.(Euphorb-iaceae)	Tree	Bark of the stem	The inner fleshy bark of the stem is crushed, boiled and soup is then drink	Diarrhoea, dysentery and acute stomach ache	Perennial
38. Pfiipfiipro (Mao)	*Drymaria Cordata* Willd (Caryophyllaceae)	Herb	Leaves	The leaves are washed and crushed The emitted flavour is inhaled	Sinus case. Also the leaves paste is used against boils and snakebite.	May to September

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of ·	Availability Season/ Periodicity
39. Mozhatobo (Mao)	*Gynura cusimba* (D Don) Moore (Asteraceae, Compositae)	Herb	Leaves and flowers	The flowers or the leaves extract are used against	Blocking bleeding during cut, injury and incident. Also the leaves are cooked along with the foodstuff of pigs against swine fever.	April to October
40. Lingmen (K)	*Lantana camara* Linn. (Labiatae; Lamiaceae)	Shrub	Leaves and young branches	The extract juice from fresh raw leaves and leaves paste is applied against	Stopping bleeding during cut/ injury/tumors. Also mixed with honey is giving in fever	Perennial
41. Khollow (Mao)	*Meriandra bengalensis* Benth. (Labiatae, Lamiaceae)	Under-shrub	Leaves	The extract juice from fresh raw leaves is prescribed against	Dysentery, Diarrhea, stomach ache, etc. The leaves paste used in the forehead is prescribed for fevers.	April to October
42. Salenipasii (Mao)	*Mussaenda roxburghii* Roxb (Melastomata-ceae)	Shrub	Leaves and root	a) The Leaves and roots are mixed and crushed into pieces. The	a) Snake bite b) Jaundice. The flowers are also prescribed as	March to July

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
				juice is then squeezed out and applied for b) The fresh leaves are warm from the fire, the juice is then squeezed out and drink	remedy for diuretic, used in dropsy, asthma and the recurrent fever.	
43 Konieu (Mao)	*Centella asiatica* (Linn) urban (*Umbelliferae, Apiaceae)*	Herb	Whole plant	The plant is washed, boiled and taken as food stuff. Also the juice extracted from the fresh plants are mixed with honey and is prescribed as remedy for	Gastric trouble, stomach ulcers, urinary troubles, digestive complaints, dysentery, etc.	March to July
44 Chaghapikhra	*Achyranthes aspera* Linn (Amaranthaceae)	Herb	Roots, Leaves and seeds	a) The roots are crushed into pieces and applied to the infected teeth/tooth b) The extract leaves juice is applied as remedy for	a) Dental caries, pyorrhea and other gum complaints. b) Irregular menstruation and piles case. Seeds are also used as remedy for hydrophobia.	Seeds- Dec. to Feb.

Local Names		Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
45	Prepribishou (Mao)	*Solanum xanthocarpum* Schrrd (Solanaceae)	Herb	Fruits and seeds	a) The seeds of this plant and dry fruits of mutleng are mixed, burn and the emitted smoke is then injected into the infected tooth/teeth b) The fruits are crushed by mixing with small amount of water and pour into the nose of cow/buffaloes	a) Dental caries b) To kill and removes the leech from the nose	Fruit-Oct to Feb
46.	Burmadiena (Mao)	*Eryngium foetidum* Linn (Umbelliferae, Apiaceae)	Herb	Leaves	a) The leaves juice are squeezed out and applied for b) The leaves are boiled and the soup is prescribed as remedy for	a) Cut/injury caused by rusting nail or broken glass b) Prevention of Titanus	March to July
47	Siiphrosii (Mao)	*Papaya carica* Linn (Caricaceae)	Tree	Fruit	The extract juice from the fruit is collected and then applied to the anus.	Piles/Hemorrhoid	September to Jan

Local Names		Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
48	Shikriisii	*Punica granatum* Linn (Punicaceae)	Shrub	Root	The roots are crushed, mixed with water and boiled The soup is then drink	Bleeding during dysentery	Perennial
49	Dziipao (Mao)	*Plantago major* Linn (Plantaginaceae)	Herb	Whole plant	The plants decoction is taken orally as a remedy against.	Dry cough.	April to Sept
50	Siidabou (Mao)	*Nicotiana tabacum* Linn (Solanaceae)	Herb	Leaves	The leaves are warm from the fire. The juice is then squeezed out and applied between the infected toes	Infection between the toes caused by germs/bacteria present in contaminated water.	May to Sept
51	Yongchak (Mao)	*Parkia rhoxburghii* Linn (Mimosaceae)	Tree	Pods and bark	The outer skin covered of the pod is taken, dry and then boiled The soup is then drink.	Gastric trouble, bleeding piles The bark extract is also giving in □iarrhea and dysentery.	Dec to Feb
52	Dalchinisii (Mao)	*Cinnamomum tamala* Linn (Lauraceae)	Tree	Leaves and bark	The leaves and bark are washed, boiled and the soup is drink Sometimes the fresh bark is taken as raw.	Rheumatism, colic disease, diarrhoea, snakebite. The leaves are also used as spices	Leaves- May to Sept.

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
53. Pekrisiibou (Mao)	*Bombax ceiba* Linn. (Bombacaceae)	Tree	Flower, root and bark	The stem bark is crushed and placed at the site of tooth ache	Dental Caries	Flower floss
54 Zhoviesii (Mao)	*Michelia champaca* Linn (Magnoliaceae)	Tree	Flowers, root and bark	a)The flower decoction is taken against b) The decoction of dried roots and bark is prescribed as	a)Stomach ache and carminative, dyspepsia, nausea, fever, etc. b)Purgative, stimulant and diarrhea	April to May
55. Letousii (Mao)	*Erythrina stricta* Roxb (Legunimosae)	Tree	Bark of stem	The bark of the stem is crushed into pieces along with the bark of kashii It is then packed into a sac and shake in the river water. The foam thus produced either killed/stunk the fishes.	Fish poison	Perennial
56. Kashin (Maram)	*Schima wallichia* Linn. (Theaceae)	Tree	Bark	The bark of the stem is crushed into pieces along with the bark of kashii.	Fish poison	Perennial

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
				It is then packed into a sac and shake in the river water. The foam thus produced either killed/stunk the fishes .		
57 Eposiibou (Mao)	*Alnus nepalensis* D. Don (Betulaceae)	Tree	Bark of the stem	The bark of the stem is crushed and boiled in water. The soup is then taken orally	Diarrhea, dysentery, stomach ache, etc	Perennial
58 Shignushi (Mao)	*Rubus ellipticus* Sm. (Rosaceae)	Shrub	Root	The roots are washed, crushed and then boiled. The soup is then drink.	Stomach ache, bleeding during dysentery and diarrhea	Perennial
59 Oramomoshi (Mao)	*Zanthophylum armatum* Linn (Rutaceae)	Shrub	Fruits	a) The decoction of stem bark is taken orally against	Carminative, stomach ache, and anthelmintic properties	Sept to Nov
				b) The fruits and seeds are used as an aromatic tonic. It is also given during	b) Fever and dysentery An extract of fruit is effective especially round worms	

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of	Availability Season/ Periodicity
60. Khroziipro (Mao)	*Solanum surattense* Burm (Solanaceae)	Herb	Leaves	The leaves are boiled or the raw leaves extract is directly applied to the anus against	Hemorrhoid/ Piles	May to Sept
61. Esiipro (Mao)	*Bergenia ligulata* (Wall) Engl (Saxifragaceae)	Herb	Leaves	The leaves are finely crushed and is then taken.	Prolong acute stomach ache.	June to Sept
62. Omeinokakiipio (Mao)ɔ)	*Acanthopanax aculeatum* Linn (Araliaceae)	Shrub	Leaves	The leaves are washed, the juice is squeezed out and mixed with small amount of water.	Stomach ache caused by charm/spell of the Witchcraft	May to Oct
63. Pfusorapro (Mao)	*Sausurea deltoidea* Linn. *(Asteraceae, Compositae)*	Herb	Leaves	The leaves are washed, juice is squeezed out and mixed with small amount of water and drink and drink.	Gastric trouble, stomachic caused by the charm/spell of Witchcraft and food poison i.e antidote	May to Sept
64 Khothou (Poumei)	*Sapindus mukorossi* Linn (Sapindaceae)	Tree	Fruit	About 5-6 mature fruits are taken in one liter of water and keep it for fermentation	Stomach ache, liver, heart and lung disorders	Sept to Nov.

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of .	Availability Season/ Periodicity
				for about 15-20 days The fermented juice is then drink.		
65 Pshibou/Pzhea shibou	*Myrica esculenta* Buch-Ham. (Myricaceae)	Tree.	Stem bark/ root	The fresh fleshy bark of the stem/root is crushed, boiled and the soup is then drink.	Diarrhea, dysentery and stomach ache	Perennial
66. Orachidu (Mao)	*Costus speciosus* Linn. (Zingiberaceae)	Herb	Stem	The stem is crushed and juice is then squeezed out About 2/3 of water is mixed with the juice and drink	Jaundice, gallstone Also 2-3 drops are applied to ear infection	Perennial
67. Monkey face (Maram)	*Jatropa curcas* Linn. (Euphorbiaceae)	Tree	Stem/branch	The juice released from cut of the stem/branches is collected and applied to the body.	a) Injury caused by the stem or fire burn, etc Note· care should be taken while handling because it is poisonous	Perennial
68. Oshikhibou (Mao)	*Lobelia stimulans* Ham. (Campanulaceae)	Herb	Tender stem	The milky juice released from the plucked leaf petioles/tender stem/branches	Tinea infection.	May to Oct

Local Names	Botanical Names & Family	Habit	Plant Parts Used	Mode of Preparation/ Extraction	Use For The Treatment of :	Availability Season/ Periodicity
				is prescribed as remedy for skin infection		
69. Ohunakury (Poumei)	*Pouzolzia bennettiana* Wight (Urticaceae)	Herb	Root	The roots are washed, crushed and applied to the body.	Cut/injury, blocking bleeding.	Annual
70 Rasopro (Poumei)	*Swertia pulchella* C.B Clarke (Gentianaceae)	Herb	Leaves	The leaves are washed, boiled and the soup is drink.	Typhoid and the malarial fevers associated with chilling and shivering.	May to Sept.
71 Letikorie (Mao) (climber)	*Crawfurdia affinis* Wall. (Gentianaceae)	Herb	Leaves and flowers	The leaves or the flowers are boiled and taken as medicinal food stuff.	Stomach ache, fever, headache, high blood pressure, etc.	Flower-Feb. to March
72. Makunamalyii (Maram)	*Desmodum triquetrum* D C (Papilionaceae)	Herb	Leaves	The leaves are washed, boiled and the soup is then drink. Also the boiled leaves are used for massaging the body.	Diarrhoea, dysentery, nerve strengthening etc.	May to Oct.

Discussion

The *Shepoumaramth* region is rich in natural resources, particularly the Non-Timber Forest Products (NTFPs) including medicinal plants. Most of the inhabitants are dependent on agriculture and forest produces. The area has been widely used by local people for centuries and in the process they evolve community codes, system and traditional values for using and conserving the forest resources which were mostly

internalise within social, economic and religious practices. However, due to population pressure on land, the forest resources are depleting at a very fast rate and in the process many rare, endangered, threatened and valuable medicinal plants are on the verge of extinction. Moreover, with the advent of the modern culture and people proneness to allopathic medicines, the valuable medicinal plants knowledge descended through generation is at the brink of extinction. Younger generation do not know the uses of the medicinal properties of the plant and their significance in human health resulting in the indiscriminate failing and slashing valuable forest species. This is a matter of great concern for the future of the people whose economy is solely based on agriculture and forest resources. Virtually not much work has been taken up in this area except few astray report. In the present study we could record as many as 72 plants of medicinal values which are used and practice by the local people. Medicinal plants of the area will provide revenue and generate employment to the local people. *Zanthoxylum armatum,* produce a good amount of fruit, seeds and bark which are very popular among the people of this area for its carminative, stomach ache and anthelmintic properties. Similar report has reported by Hertog and Wiersum (2000). In addition to this, wide spread awareness among the people of this region may help in formulating strategy for protection, conservation and management of indigenous or traditional knowledge base of plant resources for sustainable utilisation. This conservative practices will have to improve the economy of the local people and generates employment opportunities. There is still vast scope of exploration of biodiversity and conservation of bio-resources, development of data base on traditional knowledge of plant resources in this region. Method of assessing the biological properties and application of medicinal plants and finally in the development of small enterprises base on herbal medicine and cottage industries.

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Performance of Lemon *(Citrus medica)* leaves extract against *Pyricularia grisea, Aspergillus niger* and *A. flavus* pathogens associated with- rice *(Oryza sativa* L.)/ groundnut (*Arachis hypogea* L.) diseases

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Abstract

A commonly available lemon plant leaves extract in aqueous or ethanolic extract preparation and essential oil displayed fungitoxic effect against three destructive fungal pathogens, viz *Pyricularia grisea* sacc , *Aspergillus niger* and *A flavus,* inciting blast disease of rice, collar-rot and afla-root diseases of groundnut respectively. Although, ethanolic extract exhibited phytotoxicity in groundnut treatments, but the remaining two extracts proved as effective fungitoxicants, thus indicating their potential for further study and useful exploitation.

(**Keywords** *Citrus medica*/collar-rot/afla-root/ botanical extract)

Introduction

Groundnut (*Arachis hypogea),* is one of the desirable crops fitting into a rice based cropping system suited in light textured soil condition for cultivation. However, the yield of this crop is adversely affected by either of the two serious diseases *viz..* collar-rot (*Aspergillus niger)* or afla-root *(A. flavus).* The rice crop on the other hand suffers adversely due to blast disease incited by *Pyricularia grisea.* Application of synthetic fungicides in the past, have been useful to combat these diseases but, currently debated on account of its repeated use causing hazardous effect accruing to our environment. Botanical extracts being ephemeral, can serve as an alternative means to synthetic fungicide in disease management[2,3,4,5,6,9]. Present study was, therefore, envisaged at evaluating the fungitoxic effect of *Citrus medica* leaf extract against the above stated pathogens associated with groundnut and rice disease.

Materials and Methods

Extract preparations :

Green leaves of C. *medica* were plucked to weigh 100 g, washed thoroughly first in tap water, followed by sterilized double distilled water and then blot dried.

Aqueous extract :

This was prepared by crushing the chopped leaves in distilled sterilized water with 1:4 w/v in a clean pestle mortar and filtered through Whatman no. 1 filter paper. The filtrate was treated as aqueous mother extract (AME) and the residues discarded.

Ethanolic extract :

Ethanolic extract was also prepared similarly with 1:4 w/v green leaves to ethanol (95%). The extract was centrifuged at 25,000 rpm for 15 minutes, supernatant decanted into a clean sterilized beaker and the residues discarded. This extract was then treated as ethanolic mother extract (EME).

Essential oil :

Chopped green leaves were loaded in Cleavenger's apparatus and extracted in sterilized distilled water 1:1 w/v. The essential oil was collected in a clean sterilized glass voil and utilized for further studies.

These extracts were serially diluted to 0.01, 0.05 and 0.1 per cent concentration and bioassayed on a clean cavity slide against 7 day-old *P. grisea* conidia subcultured on oat meal agar (OMA) medium, previously isolated from a rice susceptible cv. HR-12. Treated conidia were incubated in humid chamber at 28 ± 2°C. Similarly, *A. niger* and *A. flavus* were isolated from the seeds of *A. hypogea* L. and subcultured on previously sterilized potato dextrose agar (PDA) medium. The mycelial growth of the three pathogens *viz., P. grisea, A. niger* and *A. flavus* were also studied at a dilution stated as above through poisoned food technique as per the method described[4] with certain modifications[7,8]. Observations on conidial germination were recorded 24 hours after incubation and on growth 7th day in case of *P. grisea* and 5th day of *A. niger* / *A. flaws* after incubation at 28±2°C. There were three replications in each treatment and control and the experiment was repeated thrice.

Results and Discussion

Aqueous, ethanolic and essential oil extracts significantly inhibited *P. grisea* conidial germination compared to control (Table 1). Maximum conidial germination (94.2%) was recorded at 0.01 per cent aqueous extract and the minimum (2.0%) at 0.1 per cent concentrations in all extracts (Table 1).

Table 1– Fungitoxicity of *C medica* leaves aqueous, ethanolic and essential oil extracts against conidial germination of *P grisea*

Concentration (%)	Extract		
	Aqueous extract	Ethanolic extract	Essential oil Extract
	Conidial germination (%)		
0 01	94 2	62 0	60 0
0.05	8 4	8 0	5 2
0.1	2 0	2 0	2 0
Control	98 0	98 0	98 0
CD 5%	2 4	2 0	3 6

All the treatments significantly inhibited mycelial growth of *P. grisea, A. niger* and *A. flavus* pathogens (Table 2). Complete growth inhibition was observed only in 0.1 per cent essential oil treatment against blast pathogen, whereas the growth in *A. flavus* and *A. niger* registered 5.1 to 5.4 cm^2, but there was no sporulation only in aflaroot incitant *(A. flavus)* of ground nut. Growth in control was 8.5 cm^2.

Groundnut seed soaking treatments, in 0.01, 0.05 and 0.1 per cent concentrations of the three preparations *(viz.,* aqueous, ethanolic and essential oil), inoculated with either *A. niger* or *A. flavus* pathogen, did significantly reduce the infection of both the pathogens at 0.05 or 0.1 per cent concentration compared to untreated inoculated control (80 - 100%). The ethanolic extract treated groundnut seed exhibited greater infection in either 0.05 or 0.1 per cent concentrations compared to the corresponding concentrations in either aqueous or essential oil extract (Table 3).

Table 2– Fungitoxicity of C *medica* leaves essential oil on mycelial growth of *P grisea*, *A niger* and *A flavus*

Concentration (%)	Pathogens		
	P grisea	*A niger*	*A flavus*
	Mycelial growth (cm^2)		
0 01	7 5	7 5	8 0
0 05	5 0	7 3	7.9
0.1	0 1	5 4	5.1*
Control	8.5	8.5	8 5
CD 5%	0.20	0.6	0 4

*No sporulation

Table 3– Groundnut seed soaking effect in C *medica* leaves aqueous, ethanolic and essential oil extracts on the infection of *A niger* and *A flavus*

Extracts/standard fungicide		*A niger*			*A flavus*		
		0 01	0 05	0 1	0.01	0 05	0 1
		Concentration (%)					
		Seed/Seedling infection (%)					
Aqueous	UNI	9.8	7.1	5 5	7 1	6 0	5 0
	INO	8 9	6 5	2.4	14 6	11.3	11 0
Ethanolic	UNI	14 9	31.1	43 3	14.9	33.4	24 3
	INO	27 6	30.4	30 3	27.9	31 2	21.9
Essential oil	UNI	4.9	1 0	1.0	5.0	3 0	1.0
	INO	20 3	16 0	12.0	29 0	20 0	17 0

Contd Table 3

Contd. Table 3

Carbendazim @2 5 g a i/kg seed	UNI	–	–	1 0	–	–	1 0
	INO	–	–	41.0	–	–	40 0
Control	UNI	1 0	1.0	1.0	20.0	20 0	20 0
	INO	80 0	80 0	80.0	100	100	100
Cd 5%	UNI	2 0	2.1	2 0	1 4	1 1	1 5
	INO	1.8	1.1	2.0	1 5	1 2	1 4

UNI = Uninoculated

INO = Inoculated with either *A niger/A flavus*

Amongst the pathogens tested, *P. grisea* was found to be most sensitive especially at the highest concentrations (Tables 1 and 2). However, groundnut seeds soaked in ethanolic extract exhibited phytotoxicity and displayed subsequently more infection (Table 3) than either aqueous or essential oil treatments. Germinating seeds displayed stunted seedling growth, chlorotic leaves and delayed radicle/plumule emergence. Contrarily, stimulatory effect due to ethanolic or essential oil extracts treatments have been reported earlier (1), though prepared from *Ocimum sanctum* leaves, hence indicating that the response of the pathogen/seed germination is specific to the type of chemical present in particular extract of specific plant.

Aqueous, ethanolic and essential oil extracts of *Citrus medica* green leaves through seed soaking were effective in reducing the collar-rot and afla-root pathogens of groundnut at all the three concentrations studied. These treatments were also effective against the blast incitant, *P. grisea* under *in vitro* conditions. However, groundnut seeds soaked in ethanolic extract preparations produced phytotoxic effect. The study thus establishes the potential of *C. medica* green leaves as an eco-friendly botanical based product to be exploited under the strategy of integrated disease management, but only after an extensive *in-vivo* evaluation trial.

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Factors influencing the production of biosurfactant and emulsification activity of *Bacillus* spp and *Pseudomonas* sp.

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Abstract

The nutritional requirements and growth characteristics of biosurfactant producing bacteria like *Bacillus* spp and *Pseudomonas* sp were determined Almost all sources of carbon used recorded high production of biosurfactants Maximum production was recorded by *Bacillus circulance* (LN-2) and sucrose was preferred as carbon source Urea or egg albumin served as inorganic or organic nitrogen source for *B circulance* (ST-I) and yclohexane for *Bacillus subtilis* (BN-l) Highest emulsification was recorded by *Pseudomonas* sp (LC-P) when hexane was used as hydrocarbon source A pH optimum of 7.0 to 8 0 was desirable for maximum production and high emulsification activity of biosurfactants The optimum temperature was observed to be 30°C for biosurfactant production

(**Keywords**: biosurfactants/*Bacillus* spp/*Pseudomonas* sp/emulsification)

Introduction

Biosurfactants are surface active compounds that can be synthesized by many microorganisms during their growth.[1] A large number of biosurfactants are synthesized by bacteria, yeast and algae. Very often, the growth of microorganism on hydrocarbon is accompanied by the emulsification of the hydrocarbon in the medium, and in most cases this has been attributed to the production of surface active compounds.[2] Interest on microbial surfactants is increasing as they can be produced in a large variety and with different properties and their production can be simplified by using inexpensive growth media. An advantage of microbial surfactants is that they are biodegradable and, hence, environmentally safe. The type, quality and quality of biosurfactants produced have been shown to be influenced by the nature of the carbon substrate, the concentration of N, P, Mg, Fe and Mn ions in the medium.[3,4,5]

This study not only confirms the above cited facts but also reports the nutritional and environmental factors favouring maximum biosurfactant production and high emulsification activity in *Bacillus* spp. and *Pseudomonas* sp.

Materials and Methods

Microorganisms and growth medium

Bacteria used in this study were *Bacillus circulance, B. subtilis, Pseudomonas* sp, *Bacillus cereus* and *Bacillus subtilis.* They were isolated from soil samples collected from Tamilnadu Agricultural University Campus, Coimbatore, Soil Salinity Research Center, Trichy and from a few automobile service stations in Coimbatore. The bacterial isolates were grown on an inorganic salts medium developed by Baruah *et al.* (1997)[6] supplemented with 1 % (v/v) cyclohexane as a carbon and energy source.

Extraction of biosurfactant

The recovery of crude biosurfactant from the cell free culture filtrate was obtained by centrifugation, evaporation and finally acetone precipitation as detailed by Cameotra (1995)[7].

Emulsification activity of biosurfactant

For quantification of biosurfactants, the crude products from each isolates was subjected to xylene emulsification assay.[8] The test was carried out by adding 5.0 ml20mM Tris buffer (pH 8.0) to a clean test tube. To this, 1.0 ml biosurfactant sample and 1.0 ml xylene were added and vortexed at high spped for 2 minutes. This was left undisturbed for 1 hour and then absorbance readings were read at 610 nm in a spectrophotometer (Schimadzu Japan Model 150) at 1 hour and 24 hours later. Higher absorbance indicates a high level of dispersion of the xylene in the buffer.

The biosurfactant production by the bacterial isolates and its activity were studied using different sources of carbon and hydrocarbons. These studies also included the effect of pH and temperature.

Results and Discussion

Nutritional requirements

Effect of carbon sources on the production and activity of biosurfactant

Table 1 shows the effect of different carbon sources on the biosurfactant production. Maximum yield was recorded by *Bacillus circulance* (LN-2), *B. cereus* (ST-l) and *B. subtilis* (LC -1) when sucrose was used and *Pseudomonas* sp (LC-P) and *B. cereus* (BN-l) when mannitol was used. The highest emulsification activity was registered by *Pseudomonas* sp and *Bacillus circulance* while using glucose and sucrose respectively. Significant production of surface active compounds by *Arthrobacter paraffeinus* ATCC 19558 was reported when the organism was grown on glucose and supplemented with hexadecane in the medium during stationary phase of growth.[9] This suggests that while one carbon source was used for the growth the other was utilized for the production of surface active compounds.

Table 1– Effect of different carbon sources on the production of biosurfactant

Carbon sources	Biosurfactant produced (g l^{-1})				
	Bacterial isolate				
	LN-2	LC-1	LC-P	ST-1	BN-1
Glucose	5.13	5.31	3.50	2.73	3.94
Sucrose	5.76	5.53	1.70	5.62	3.20
Lactose	4.11	5.02	LOO	2.96	3.11
Mannitol	2.49	3 25	4.3g	4.20	5.46
Starch	1.84	3.24	2.73	4.16	3.10

The effect of different hydrocarbons like cyclohexane, hexane, heptane, xylene and kerosene on the production and activity of biosurfactants has been studied (Table 2 and Fig. 1). Maximum production was recorded by all the isolates when cyclohexane served as source of carbon. *Pseudomonas* sp and *Bacillus subtilis* registered highest emulsification activity when hexane was used. Some bacteria produce surface-active agents when grows with water soluble or water insoluble substrates, although in the latter case production was higher. *Pseudomonas aeruginosa* 44T1 produced rhamnolipids when grown on C_{12} n-alkanes or olive oil but not on hexadeeanc or a mixture 01 long chain n-alkanes[10], It is perhaps the chain length of hydrocarbon substrates that decided the production.

Table 2– Effect of different hydrocarbons on the production of biosurfactant

Hydrocarbons	Biosurfactant produced (g l^{-1})				
	Bacterial isolate				
	LN-2	LC-1	LC-P	ST-1	BN-1
Cyclohexane	5 12	5.56	6 38	5 54	6 53
Hexare	480	4 05	3 95	5 30	4 50
Heptare	4 65	3 66	4 37	4 07	3 32
Xylene	3 87	5 41	4 00	4 76	3 36
Kerosene	4 88	3 75	4 33	2 77	1 63

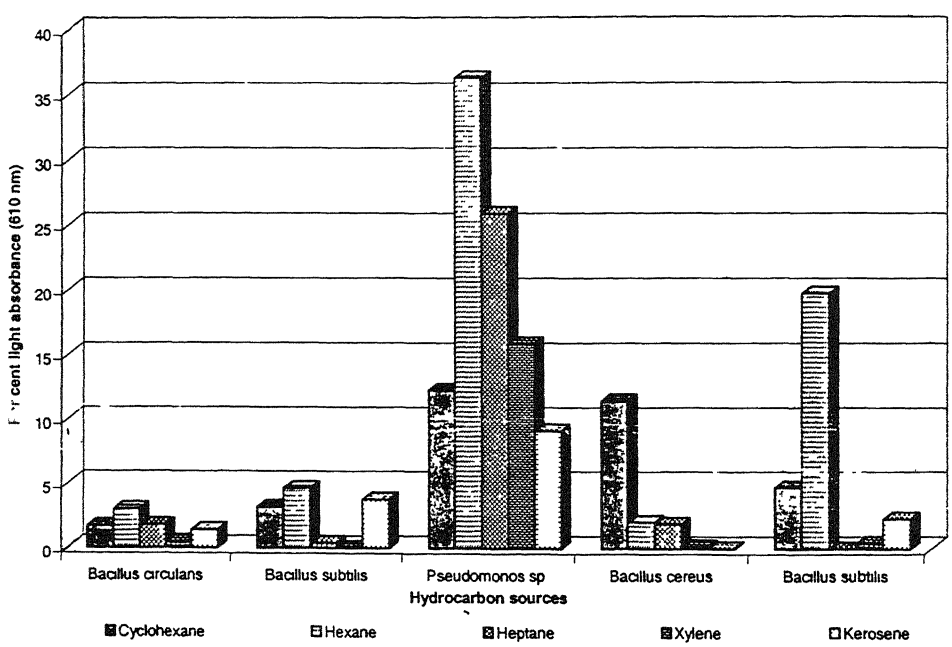

Fig 1– Effect of different hydrocarbons on emulsification activity of biosurfactant

Effect of organic and inorganic sources of nitrogen on the production and activity of biosurfactant.

Results on the effect of different inorganic and organic nitrogen sources are shown in Tables 3 and Fig. 2. With urea as nitrogen source, high biosurfactant yield was obtained by all the isolates. The highest activity was shown by *Bacillus subtilis* (BN-I) with diammonium phosphate. A higher biosurfactant production was observed by Ochsner *et. al.*[1] in *Pseudemonas aeruginosa* strain PG 201 with nitrate as sole nitrogen source. Since nitrogen is, macronutrient for microbial growth, adequate nitrogen supply is essential for biomass formation. On the other hand, nitrogen starvation often encourages product formation.

Table 3– Effect of different inorganic, nitrogen sources on the production of biosurfactant

Inorganic nitrogen sources	Biosurfactant producee (g l^{-1})				
	Bacterial isolates				
	LN-2	LC-1	LC-P	ST-1	BN-1_1
Urea	4 99	4.10	3.17	6 89	5 66
Sodium nitrate	1.83	5 28	3.92	6 30	4.61
Potassium nitrate	2 29	4 20	5.30	6.39	4 51
Ammonium chlorice	3.01	4.30	3 10	1.91	3.49
Diammonium phosphate	3.90	4.35	4.90	5 98	3.57

The study further revealed that egg albumin served as the best organic nitrogen source for all. the isolates; however, high production was observed by *Pseudomonas* sp and *Bacillus cereus.* Biosurfactant activity was found to be high when yeast extract was used by *Pseudomonas* sp and glutamic acid by *Bacillus circulans.*

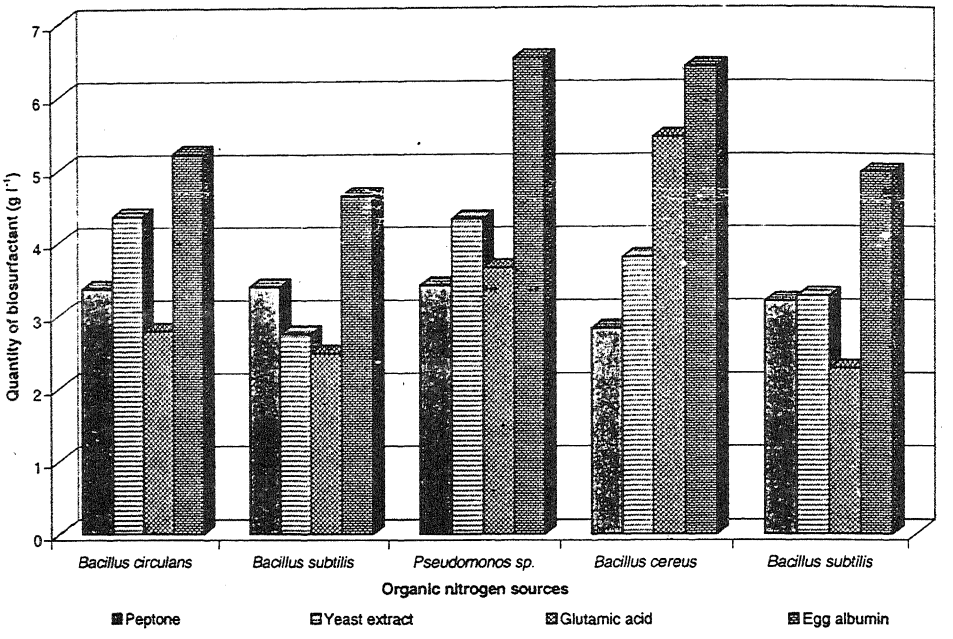

Fig. 2– Effect of different organic nitrogen sources on the production of biosurfactant

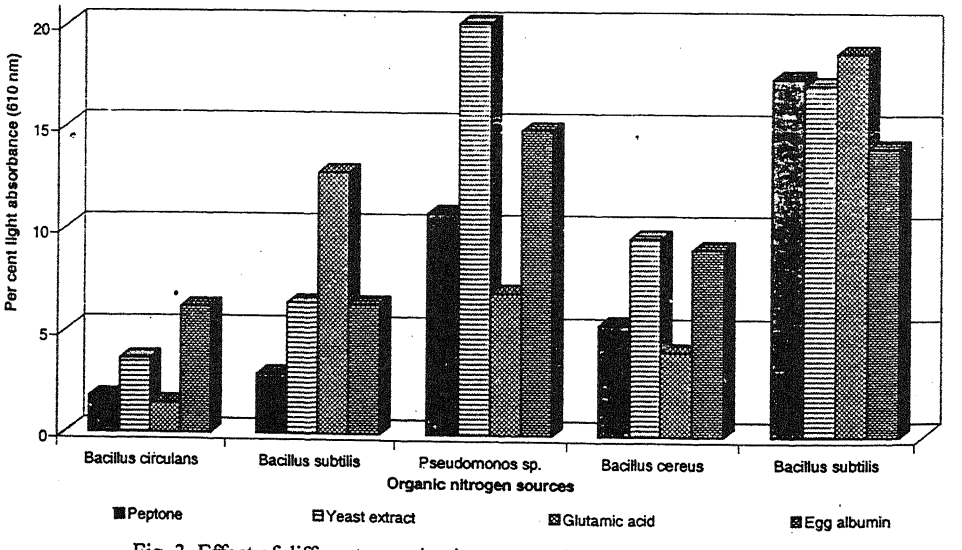

Fig. 3–Effect of different organic nitrogen emulsification activity of biosurfactant

The effect of initial pH of the medium on biosurfactant production and activity is shown in Table 4. The highest production and emulsification activity were obtained with pH range of 7.0 - 8.0 by all the bacterial isolates. *Pseudomonas* sp showed a very high activity at pH 8.0. Biosurfactant production and emulsification index measurements were less influenced by the cultivation pH since the biosurfactant production and activity of *Rhodococcus* fell within a pH range of 6.5 - 7.2.12 Also lowering the pH to below 5.5 decreased both growth and biosurfactant production of *Bacillus* sp.[13]

Table 4– Effect of different pH on the production of biosurfactant

pH	Biosurfactant producee (g l^{-1})				
	Bacterial isolates				
	LN-2	LC-1	LC-P	ST-1	BN-1
5.0	4 63	4 97	4.31	3 23	5 28
6 0	5 32	3.83	4.03	5 01	4.83
7.0	6.09	7.24	5.84	5.68	7.69
7.5	7.32	6 59	5.15	7 73	7.97
8 0	5.67	6 54	6.36	7 14	7 23

Effect of temperature on the production and activity of biosurfactant

At 30°C, the production and activity of biosurfactant was maximum for all the bacterial isolates (Table 5). *Bacillus circulance* (LN-2) registered maximum yield and activity. *Pseudomonas* sp showed no production and hence no activity at 45°C. Lower or higher temperatures generally had a depressing effect on both production and activity as detected by lower cell yields.

It is well established that the physiology of a microorganism is not exclusively determined by it's genetic makeup, but is also a function of the environmental conditions.[14]

Table 5– Effect of different temperature on emulsification activity of biosurfactant

Incubation Temperature (°C)	Per cent light absorbance (610 nm)*				
	Bacterial isolates				
	LN-2	LC-1	LC-P	ST-1	BN-1
5 0	4 63	4 97	4 31	3 23	5 28
6 0	5 32	3 83	4 03	5 01	4 83
7 0	6.09	7 24	5 84	5 68	7 69
7 5	7 32	6 59	5.15	7 73	7 97
8 0	5 67	6.54	6 36	7 14	7 23

* -10^{-2} dilution of original emulsification

The nutritional and cultural conditions for high biosurfactant production with these organisms represent a realistic of the feasibility of bulk production of biosurfactants. Studies on biosurfactants have opened up new area of microbial utility for mankind. It is hoped that microbial surfactants would soon replace the presently available chemical surfactants. Much of the earlier studies have indicated the potential of different species of bacteria for producing biosurfactants; however, this paper describes the ideal nutritional and environmental conditions that favours the production of biosurfactants.

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Analysis of asarones from commercial samples of *Acorus calamus* L.

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Abstract

The presence of asarones in the commercial samples of *Acorus calamus* provides a potent danger to human life as the rhizome of this plant is extensively consumed in India as an Ayurvedic medicine Alcoholic extract, aqueous extract or the raw drug itself from the rhizome of Acorus is presently being used in the several formulations which are consumed by millions of young and old people From our study it was evident that only the alcoholic extract when used contains asarones while the aqueous extract does not contain detectable levels It is thus advisable that manufacturers of Ayurvedic medicines using *Acorus calamus* use only the aqueous extract for manufacturing their formulation

(**Keywords** *Acorus calamus*/asarone/ayurved)

Introduction

Roots and rhizomes of *Acorus calamus* L. is commonly used in several Ayurvedic formulations for the treatment of many mental disorders such as insanity, insomnia, melancholia, epilepsy, neurasthenia and hysteria[1]. Baxter *et. al.*[2] isolated two pure chemical principles from the oil of *Acarus calamus* L. namely α-asarone and β-asarone. Further studies by Dyer *et. al.*[3,4] revealed the presence of these asarones in alcoholic beverages like wines and flavors. In Europe, a special committee of Experts of the Council of Europe and the EEC has recommended limit for asarones in beverages and foodstuffs to 1mg/kg[5]. Medicines Control Agency (MCA) of UK reported β-asarone isolated from calamus oil as a potent carcinogen, nephrotoxic and causes convulsions in humans[6]. In spite of the several medicinal properties of *A. calamus*, The Food and Drug Administration (FDA) has prohibited the use of this plant in any form such as the root, extract or oil, in any food or drug as a result of the toxicological studies, where the asarones have been implicated as toxins[7]. It is

therefore important to detect for the presence of asarones in the commercial root powder. During the course of this study, formulations of leading brands where *A. calamus* was used as an ingredient were also checked for the presence of asarones.

Materials and Methods

Fresh rhizome samples of *A. calamus* collected from different sources were identified using their macro and microscopical characters as outlined in Ayurvedic Pharmacopoeia of India[8]. All the rhizomes were diploid in nature and there was no difference in anatomical features. They were washed thoroughly and shade dried for extraction.

The shade-dried samples were extracted separately with 100% Methanol and Pure Reverse Osmosis water (Millipore Water Purification System, France) in a Soxhlet apparatus with refluxing for eight hours at 55°C. The extracts were filtered through 0.2 micron nylon filter membrane and injected into the HPLC system. An Ayurvedic preparation, Learnol Plus which is being widely used against mentally challenged children was also checked for the presence of asarones. This product is given as syrup and was diluted with 100% methanol and Pure RO Water separately and injected into the HPLC system (Shimadzu, Japan, LC 8A, dual pump, Deuterium lamp). Standards of α- and β-asarone were purchased from Sigma, USA for estimation of the asarones in the samples. The LD_{50} values of α- and β-asarones are 300mg/kg and 122mg/kg i.p. in mice respectively.

Methanolic (100%) and aqueous extracts were analysed for the presence of α- and β-asarones from samples of *A. calamus* obtained from New Delhi, Varanasi, Coimbatore in India and the popular Ayurvedic Syrup Leamol Plus manufactured by Herbal Specialities Limited, Guwahati, India, using a modified procedure of Tsai *et. al.*[9] for the estimation of α-asarone and β-asarone. Phenomenex column ODS C18 (250mm x 4.6mm), 5μ particle size was used against acetonitrile: water: triethylamine (50:50:0.1 v/v, pH 5.5, adjusted with orthophosphoric acid) as the mobile phase. Asarones were detected at 257 nm using a UV detector. The approximate retention time of β-asarone was found to be 23min and that of α-asarone was 26min.

Results and Discussion

There are several studies done earlier on the extracts of *Acorus* in experimental animals. The physical and chemical properties of oil of calamus of Indian, Javanese, European, North American, Japanese and Russian origins have been described. LD_{50} of volatile oil from *Acorus* was assessed by Dandiya *et. al.*[10] and it was found to be

0.12 to 0.22g per kilogram of mice. Kelkar and Rao[11] concluded that the difference in action recorded between the Indian and other commercial varieties of calamus oil was not due to the presence of any new constituents but due to the predominance of asarone in the Indian origin. The Indian oil has been reported to contain 82% asarone while the other commercial varieties have approximately 7%.

The methanolic extracts of the rhizomes collected from three regions namely, Coimbatore (South India), New Delhi (North India) and Varanasi (North India) showed the presence of α- and β-asarones (Table 1). The ratio of asarones indicated that β-asarones were present more than α-asarones in those samples. Rhizome A from Coimbatore showed the highest amount of asarones and Rhizome C from Varanasi showing the lowest. Both *a*- and β-asarones were not detected in methanolic and aqueous extracts of Learnol plus syrup, one of the commercial Ayurvedic preparations taken for the study.

Table 1– Percentage of asarones in the samples

Samples	100% Methanolic extract		Aqueous extract	
	β-asarone (in ppm)	α-asarone (in ppm)	β-asarone (in ppm)	α-asarone (in ppm)
#Rhizome - A	51714	1020	$ND	ND
Rhizome - B	48137	920	ND	NO
Rhizome - C	45624	880	ND	ND
*Syrup (10%)	ND	ND	ND	ND
Syrup (25%)	ND	ND	ND	ND
Syrup (50%)	ND	ND	ND	ND

#Rhizome A from Coimbatore, Rhizome B from New Delhi and Rhizome C from Varanasi were obtained for the study.

*Syrup-Learnol plus was obtained from Herbal Specialties Limited, New Delhi.

$ ND - Not detected

A leading brand (tablet) that has *Acorus calamus* as one of the ingredient is currently being used for mental deficiency in children contains asarones in higher amounts (data not given). Asarones are water insoluble hence it might be useful if statutory authorities who regulate such formulations in India make it mandatory for manufacturers of such drugs that any formulation which contains *Acorus calamus* should use only the aqueous extract.

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Chlorogenic acid content and specific activities of related enzymes in potato plant tissues infected by late blight fungus *Phytophthora infestans*

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Abstract

A rapid increase in the chlorogenic acid content after infection by the late blight fungus, *Phytophthora infestans* was noticed during early period of infection of leaf as well as tuber tissue of four potato cultivars showing differential susceptibility to infestation The healthy leaves of the tolerant cultivars contained significantly higher level of chlorogenic acid The activities of both polyphenol oxidase and peroxidase enzymes increased after infection in all the cultivars. Reduction in the level of enzyme activity, however, was observed after longer period of infection, in both tuber and leaf tissues of all the infected cultivars

(**Keywords** : potato plant tissues/ chlorogenic acid/ polyphenol oxidase/ peroxidase/ *Phytophthora infestans*

Introduction

Phenolic compounds have long been correlated with disease resistance in response to infection by plant pathogens[1,2]. Chlorogenic acid, an ortho-dehydroxy phenol has been claimed to be present in variable quantities in healthy and infected plant tissues[3]. The enzymes participating in the synthesis of phenolic compounds, particularly polyphenol oxidase and peroxidase are also known to play important role in the host parasite Interaction.

The pesent communication deals in the changes with respect to chlorogenic acid, polyphenol oxidase and peroxidase in resistant and susceptible cultivars of potato during infection of late blight.

Materials and Methods

Cultivar : The following cultivars of potato *(Solanum tuberosum* L.) were analysed for the chlorogenic acid content and activity of enzymes like polyphenol

oxidase and peroxidase: Kufri-Jyoti (resistant), Kufri-Khasigaro (moderately resistant), Phulwa and Up-to-date (both susceptible).

Pathogen : Race 4 of *Phytophthora infestans,* supplied by the Station Director; Central Potato Research Institute, Shillong, Meghalaya, was used for inoculation of tuber and leaf tissues of potato.

Preparation of sample : Healthy freshly harvested tubers of 60 day old plants of all the four cultivars of potato and the leaflets of 3rd to 6th leaf from the apex plant of the same age group were carefully collected and were used for chemical analysis. Plant tissues were inoculated with race 4 of *P. infestans* as per procedure adopted by Lapwood[6] by placing a droplet of known size containing 50,000 sporangia/ml on the leaf and tuber surface. After incubation of 7, 10, 13 and 16 days at 20 + 1°C, circular disks of the infected regions were cut and used in the experiments.

Estimation of chlorogenic acid : The methods proposed by Johnson and Schaal[7] was adopted for the estimation of chlorogenic acid. Absorbancy of the sample was read in 'Bauch & Lomb Spectronic-70' colorimeter at 515 run. The chlorogenic acid was calculated from a standard curve prepared with catechol.

Estimation of enzymes : The enzyme extraction was carried out according to the method described by Gupta *et al.*[2] One gram of finely chopped fresh leaves and tuber samples were ground with sand in a previously chilled mortar with 4-5ml of 0.01M phosphate buffer (pH 7). The homogenates were strained through four layers of cheese cloth. The filtrates were centrifuged at 10,000 x 1g for 20min at 4°C and the supernatant was used for enzyme assays. Three separate extractions were made for each sample. Polyphenol oxidase(PPO) and peroxidase(PO) were assayed according to Horwitz *et al.*[8] and Seevers *et al.*[9] respectively. Enzyme units were expressed as changes in 0.1 absorbance min^{-1} mg^{-1} protein.

Results and Discussion

Results presented in Table 1 show significant variation in the level of chlorogenic acid in the tuber as well as leaf tissues as a result of infection. In all the cultivars, there was a sudden increase in the level of chlorogenic acid during initial stages of infection and then at a slower rate during later period. Increase in the content was much more significant in the infected leaves of the cultivars Kufri-jyoti and Kufri-Khasigaro. Increased chlorogenic acid content after infection has also been reported by Friend *et al.*[3] in 'Majestic 'and 'Orion' cultivars of potato.

Results also indicate variation in the level of chlorogenic acid in different cultivars showing differential susceptibility. The healthy leaves of the cultivars Kufri-Jyoti and Kufri-Khasigaro contained significantly higher amount of chlorogenic acid than the leaves of the other two cuitivars. The healthy tubers of all the four cultivars however, did not reveal any significant variation. The result of the study that chlorogenic acid content can not be directly associated with resistance mechanism of potato plants so far tuber tissues are concerned, while positive correlation was noted between chlorogenic acid level of leaf tissues and resistance to infection by *Phytophthora inJestans.*

Table 1– Chlorogenic acid content (mg/100g) of potato plant tissues in relation to infection by *Phytophthora infestans*

Cultivar	Healthy		Infected							
			7 Days		10 Days		13 Days		16 Days	
	T	L	T	L	T	L	T	L	T	L
CV 1	19.67	108.66	29 21	132 58	33 33	136 24	33 67	136 86	37.23	139 02
CV 2	21 00	92.24	26.42	128.57	29 45	131.65	31 95	134 10	14 00	137.67
CV 3	17.95	62.00	21 63	76 35	26.32	84 25	26 71	92.00	27 65	94 23
CV.4	18.33	58.00	24.44	67.00	26.10	70 33	26 50	70 65	28.42	71.41

C D. for cultivar at 1%=1.67

T=Tuber tissue

L=Leaf tissue

Figures are av of 3 replicates

CV .l= Kufri-Jyoti; CV.2= Kufri-Khasigaro

CV.3= Phulwa, CV.4= Up-to-date

The level of activity of polyphenol oxidase (Table 2) increased in the infected tubers and leaves of all the cultivars, maximum increase was observed in the leaves of the resistant cultivars. Further, increased activity of PPO was also recorded in the uninoculated leaves of the resistant cultivars indicating involvement of this enzyme in resistance mechanism of the tissues. Sokolova *et al.*[10], stated that the defence action of chlorogenic acid may not be due to its direct toxicity but for substances produced under the influence of host polyphenol oxidase possibly caffeic and quinic acids and their derivatives.

The specific activity of peroxidase (Table 3) was several times higher than that of polyphenol oxidase. Activity of PO increased rapidly at the initial stage of infection in both tuber and leaf tissues of all the cultivars but gradually declined after longer

period of incubation. The reduction in PO activity was more prominent in the tissues of the resistant cultivar Kufri-Jyoti. The leaves of the cvs. Phulwa and Up-to-date showed increase in PO activity during later period of infection. Increased PO activity was also prominent in both healthy leaf and tuber tissues of cultivars Kufri-Jyoti and Khasigaro, which are resistant to infection, as compared to the other two susceptible cultivars, suggesting a possible correlation of peroxidase with resistance mechanism of potato plants.

Table 2– Specific activity of polyphenol oxidase (expressed as change in 0 1 absorbance min^{-1} mg^{-1} protein) in tuber and leaf tissues of potato plants in relation to infection by *P infestans*

	Healthy		Infected							
			7 Days		10 Days		13 Days		16 Days	
	T	L	T	L	T	L	T	L	T	L
CV.1	3.06	5.21	5.75	8.32	4.92.	8.15	4.96	7 85	4.87	7 72
CV.2	2.24	4.30	3.92	7.96	3 92	7.90	3.78	6 74	3.80	6.86
CV.3	2.21	3.18	2.34	4.30	2.40	4.72	2.40	4.00	2 30	4 12
CV 4	2.24	1.20	2.38	3.35	2.38	3.35	2 41	3.40	2.28	3 30

C.D. for cultivar, at 1 %= 2.76

T= Tuber tissue

L= Leaf tissue

Figures are av of 4 replicates

CV .l= Kufri-Jyoti, CV.2= Kufri-Khasigaro

CV.3= Phulwa; CV 4= Up-to-date

Table 3– Specific activity of peroxidase (expressed as change in 0.1 absorbance $min^{-1}mg^{-1}$ protein) in tuber and leaf tissues of potato in relation to infection by *Phytophthora infestans*

	Healthy		Infected							
			7 Days		10 Days		13 Days		16 Days	
Cultivar:	T	L	T	L	T	L	T	L	T	L
CV. 1	154.6	233.7	572.0	790.0	581.3	798.6	568.4	784.0	554.4	687 5
CV. 2	173.0	234.6	581.4	765.0	589 0	768.0	564.2	732.0	564.2	730 0
CV. 3	54.3	86.7	271.4	236.0	271.4	247.5	256.5	255.0	243.0	255.4
CV. 4	71.5	80.3	231.3	205.0	234.5	237.0	216.4	225.0	209.5	224.5

C.D. for cultivar at 1%=0.06

Figures are av. of 4 replicates

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Effect of sugars on the growth of callus in *Aquilaria agallocha* Roxb. (Thymeliaceae)

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Abstract

Callus of *Aquilaria agallocha* Roxb. initiated in MS + 2,4-D (6mg/l) + Kn (2 mgl/l) was grown in MS medium supplemented individually with different sugars viz. sucrose, fructose, glucose and maltose, each in various concentrations ranging from 15 to 75 g/ 1. Fructose at 30 g/l showed the highest growth rate of 547.25 percent increase in fresh callus biomass recorded after 45 days of culture. Maltose showed the least growth rate, which showed a highest 212.76 percent increase in fresh cell biomass in the concentration 60 g/l after 45 days of culture.

(**Keywords :** sugars/callus/ *Aquilaria agallocha* Roxb.)

Introduction.

Aquilaria agallocha Roxb. is an evergreen tree belonging to the family Thymeliaceae. In India it is distributed in almost all the areas of the North-eastern region and is mostly found in the hills of Arunachal Pradesh, Nagaland, Manipur Mizoram and Tripura. In the plains it is mostly found in upper Assam districts and Barak valley. It is also distributed in several Southeast Asian countries viz. Thailand, Burma, Bangladesh and Bhutan[1].

A. agallocha is of great commercial and economical importance due to an aromatic oil that accumulates in its wood and obtained by hydro-distillation. Agar oil is an excellent perfume retainer and highly prized by European perfumers for mixing with their best grades of scents[1]. Agar laden wood chips are also widely marketed. The oil also has certain medicinal values. It has been used as a stimulant, cordial tonic and carminative and enters into several compound preparations. In Malaya it is also used as cosmetics and liniment in various skin diseases. The tree is heavily exploited particularly for its oil and so it has become rare in the natural habitat. The natural propagation of the tree is through seeds, which remains viable for about a week. So to reduce the pressure on the standing tree alternative methods by scientific means to produce the oil from callus tissue is an open option. For this production of large bulk

of callus is necessary. Attempt has therefore, been made to increase the production of callus biomass by manipulating the culture medium. In this paper effect of some sugars in rapid production of callus has been reported.

Materials and Methods

Fresh explants consisting of leaf, stem and shoot tip of *A. agallocha* Roxb. were selected for *in vitro* callus initiation. MS medium[2] supplemented with 2,4-D and kinetin were effective in inducing callus[3]. For callus initiation MS medium was supplemented with sucrose (30 g/l) as carbon source and 2, 4-D (0.5-8.0 mg/l)+ kn (2 mg/l) as hormones. The callus was multiplied by continuous subculture in fresh media with similar composition. 45 days old secondary callus maintained in MS medium supplemented with 2,4-D (6 mg/l) + Kn (2 mg/l), was used as inoculum. To study the effect of sugars on the callus growth, 2.0 g of white and friable callus was inoculated into MS-basal medium supplemented individually with sucrose, fructose, glucose and maltose and each of them in various concentrations (15, 30, 45, 60 and 75 g/l.) Gelling of the media was done with bacteriological grade agar (0.9%). Sterilization of the media and inoculating instruments were done at 15 lb pressure for 15 minutes.

Fresh explants were first washed with detergent solution (Tween-20), followed by surface sterilization with mercuric chloride (0.1 %) and ethanol (70 %), and finally rinsed several times in sterile double distilled water. Explants were cut into desirable sizes before inoculating into the medium. The whole operation was done aseptically under a laminar air flow cabinet. Data were recorded in terms of the following parameters- time taken for callus initiation in days and fresh weight of callus biomass after 105 days of explant inoculation. Calli were maintained in MS + 2,4-D (6 mg/l) + Kn(2 mg/l) by regular sub culturing after 45 days. Initial green color of the callus was lost due to continuous sub culturing and turns white and fragile. These white calli were used to study the effect of sugars on callus growth. Previously established callus was inoculated into 100 ml medium taken in 250 ml conical flasks. Callus biomass produced at different intervals (15, 30, 45 and 60 days) were recorded for studying the rate of growth, which is expressed in percentage increase of fresh cell biomass. The cultures were maintained under cool fluorescent light intensity of 2000-3000 lux for 16-8 hours light-dark period and temperature of 25 ± 2°C.

Results and Discussion

Leaf was found to be better among all the explants, in terms of time taken for callus initiation and amount of biomass produced. The time taken for callus initiation

varied from 25 to 32 days in leaf, 28 to 34 days in stem and 32 to 36 days in shoot tip. Callus biomass production was found to vary among the different explants as well as the treatments which has been recorded after 105 days of culture. In case of leaf, fresh biomass varied from 6.425 to 8.890 g, while in stem and shoot tip it varied from 6.020 to 8.200 g and 5.890 to 7.625 g respectively. Leaf callus grew comparatively more rapidly than calli from other explants . Two distinct type of calli were obtained from the explants of *A. agallocha.* Initially the callus was greenish to greenish yellow in color and partially compact. But after continuous subculturing of the callus, the green color is lost and the callus turned white, after 5-6 months. The white callus was loose and friable having faster growth rate, compared to green callus.

Among the different sugars taken for the study fructose was found to be the best sugar for callus growth of *A. agallocha.* It supported the highest growth rate with a 547.25 percent increase of fresh callus biomass, where as the corresponding figure in sucrose containing medium was found to be 475.62 % increase after 45 days of growth. Maltose was found to be least effective as there was only 212.76 % increase of fresh cell biomass after similar period of growth recorded in the concentration 60 g/l. Callus growth rate was also high in glucose containing medium and 423.66 % increase in cell biomass was recorded in glucose-30 g/l. As far as the concentrations of the sugars is concerned, good growth rate was generally found to be in the concentrations ranging from 30 to 45 g/l. However as the concentrations of sugars were increased, a declining trend of the callus growth rate was observed in almost all the sugars. However in case of fructose a slight better growth was seen at 75 g/l concentrations, compared to that in 45 and 60 g/l concentration. In fructose concentration of 75 g/l there was 502.21% increase of fresh cell mass after 45 days as against 466.21 % and 352.00 % in 45 g/l and 60 g/l concentrations respectively (Table 1).

The combined effect of 2,4-D and kinetin is essential for the initiation of callus in *A. agallocha* Though a range of concentration of 2,4-D varying from 0.5 to 8.0 mg per litre of culture medium was effective in presence of 2.0 mg/l kinetin, a combination of 6 mg/l 2,4-D + 2 mg/l kn was most effective in the initiation of callus and total biomass production[3]. Several workers have demonstrated the requirements of both 2,4-D and kinetin in the initiation of callus in several woody species, viz. *Sesamum indicum*[4], *Phoenix dactylifera*[5], and *Ceratozamia hildae*[6]. MS medium supplemented with 5 mg/l 2,4-D and 0.01 mg/l kn was used to initiate callus in *Terminalia arjuna* as reported by Nishi Kumari *et al*[7]. Lower levels of 2,4-D (2 mg/l) and kn (0.5 mg/l) were used in modified MS medium for induction of callus in *Havea brasiliensis* by Jayshree *et al*[8]. Loss of chlorophyll during subculturing is observed in

A. agallocha callus. The pattern of growth of the callus is very similar to that of other plants[9], and shows a typical sigmoid curve. The highest growth of the callus was found to be between 15 to 45 days of incubation after which senescence and cell death starts.

Table 1– Effect of different sugars on callus growth (expressed in % increase over cell biomass in *A agallocha*

Sugars g/l	Percent increase in cell biomass			
MS+	15 days	30 days	45 days	60 days
MS+O	-	20 00	32 00	30 00
MS + Sucrose-15	80 66	213.67	383.50	368 18
MS + Sucrose-30	89 67	259.91	475 62	438 35
MS + Sucrose-45	89.58	272.61	485 55	467 00
MS + Sucrose-60	82.66	158.43	300 54	338.35
MS + Sucrose-75	74.54	190 64	314 00	242.65
MS + Glucose-15	68.76	182 87	284.11	281 56
MS + Glucose-30	79.54	280.66	423.06	364 58
MS + Glucose-45	61.94	176.22	320.90	290.62
MS + Glucose-60	55 92	195 23	278 52	282.70
MS + Glucose-75	58.62	75 32	167.04	152 58
MS + Fructose-15	112 33	288 04	485.92	426 87
MS + Fructose-30	150 05	393.84	547 25	482 05
MS + Fructose-45	160 33	342 64	466.21	462.02
MS + Fructose-60	97.61	258.00	352 00	300 29
MS + Fructose- 75	87.42	309 09	502 21	431.22
MS + Maltose-15	47.18	98 46	187.43	132.00
MS + Maltose-30	56.98	140.46	192.55	143.98
MS + Maltose-45	48.96	129.43	180.66	176.98
MS + Maltose-60	56.45	96.00	212.76	160.60
MS + Maltose-75	40.67	85.00	185.98	170.76

Effect of different sugars in tissue culture of various plants have been reported by several workers. Monosaccharides such as arabinose and xylose, disaccharides like cellobiose, maltose and trehalose all of which are capable of being broken down to glucose and fructose can be sometimes used as partial replacements of sucrose[10]. Besides these raffinose, mannose and lactose were also used in tissue culture of certain plant species[11]. Good growth with fructose is reported in several plant species. Fructose was found to be best in the production of adventitious shoots from *Glycine max* cotyledonary nodes especially if the concentration of the nutrients salt supplied was inadequate[12]. When sucrose was replaced by fructose 30 g/l shoot and leaf growth and axillary shoot formation was found to be stimulated in *Castanea*[13]. Some orchid species were reported to grow better on glucose[14,15,16]. Beneficiary effect of glucose was reported in *Alnus crispa, A. Chordata* and *A. rubra* shoot culture while sucrose was found to best in *A. glutinosa,*[17,18,19]. Gautheret, 1945[11] found sucrose to be best carbon followed by glucose, maltose and raffinose in carrot tissue culture. However ftuctose was less effective and mannose and lactose was found to be least effective.

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chromosomal abnormalities were calculated for all the doses, which are considered as parameters for evaluating the relative cytotoxicity of the pesticides. The mitotic index is expressed in term of relative division rate (RDR) while chromosomal abnormality in terms of relative abnormality rate (RAR).

$$RDR = \frac{\textit{Percentage of dividing cells in treated variant} - \textit{Percentage of dividing cells in control variant}}{100 - \textit{Percentage of dividing cells in treated variant}} \times 100$$

$$RAR = \frac{\textit{Number of abnormal cells}}{\textit{Number of cells observed}} \times 100$$

Results

Mitotic division was normal in control roots (Figs. 1-4). The mitotic index of the untreated roots was 20.57 ± 0.07 whereas in roots treated with different concentrations of aldrin it ranged from 9.13 ± 0.02 (1.0%) to 17.76 ± 0.17 (0.1%), with malathion from 8.02 ± 0.05 (1.0%) to 16.98 ± 0.09 (0.1%) and with monocrotophos from 7.43 ± 0.10 (1.0%) to 16.03 ± 0.09 (0.1 %). The RDR was considerably decreased however the RAR was increased along with the increasing concentration of the pesticides (Table 1). Increase in the negative value of RDR was directly proportional to the severity of the mitotic inhibition. It was noticed that all the pesticides had almost similar type of mitodepressive effect as compared to the control (Table 1).

Maximum chromosomal abnormalities were observed at 1.0% treatment of monocrotophos (i.e. 6.16 ± 0.14) and minimum at 0.1% aldrin treatment (i.e. 2.65 ± 0.05). The percentage of mitotic abnormalities ranged from 2.65 ± 0.05 - 4.94 ± 0.10%, 2.94 ± 0.08 - 5.68 ± 0.10% and 3.21 ± 0.13 - 6.16 ± 0.14% during the treatment of aldrin, malathion and monocrotophos, respectively (Table 2). Observations clearly suggested that the monocrotophos was able to induce maximum mitotic abnormalities and it was subsequently followed by malathion and aldrin. Fragments were evidently formed as a result of chromatid and subchromatid breaks. Though, these were observed at all the doses of pesticides but reached to maximum (1.07 ± 0.08%) at 0.5% concentration of monocrotophos and minimum percentage (0.41 ± 0.03%)was observed at 0.1 % concentration of aldrin.

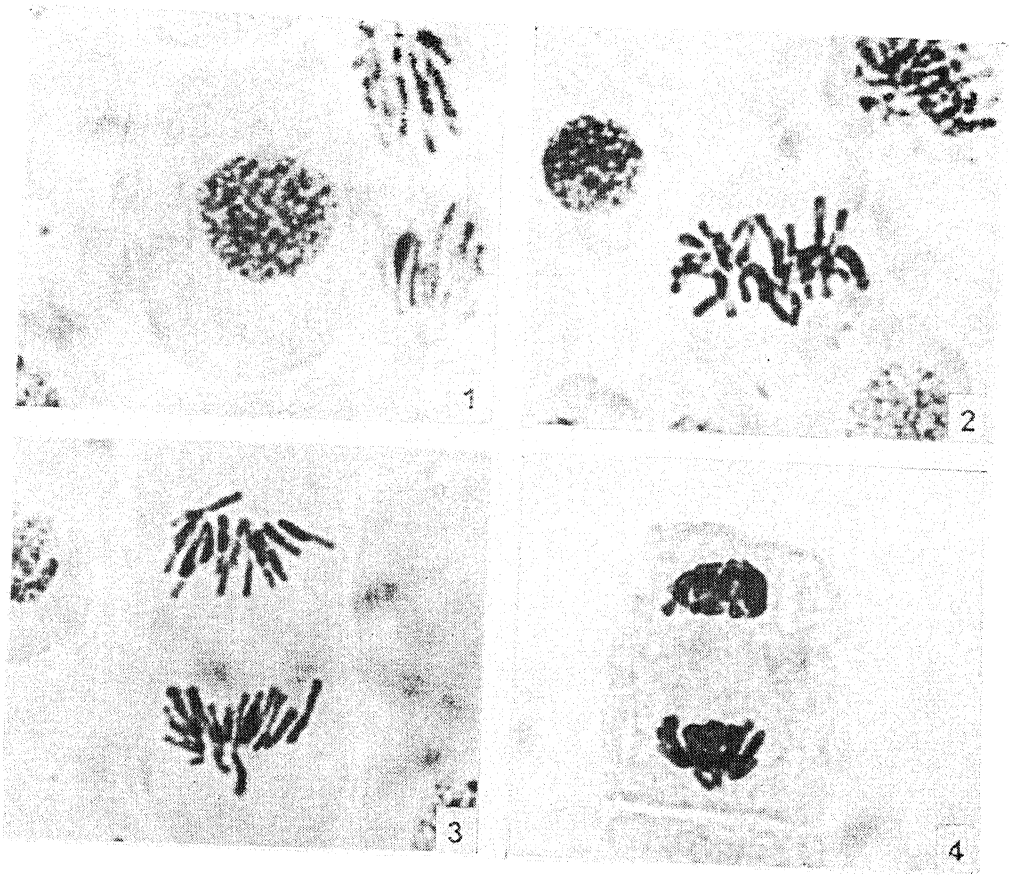

Fig. 1-10– *Allium cepa*. Fig. 1– Normal prophase. Fig. 2– Normal metaphase. Fig. 3– Normal anaphase Fig. 4– Normal telophase.

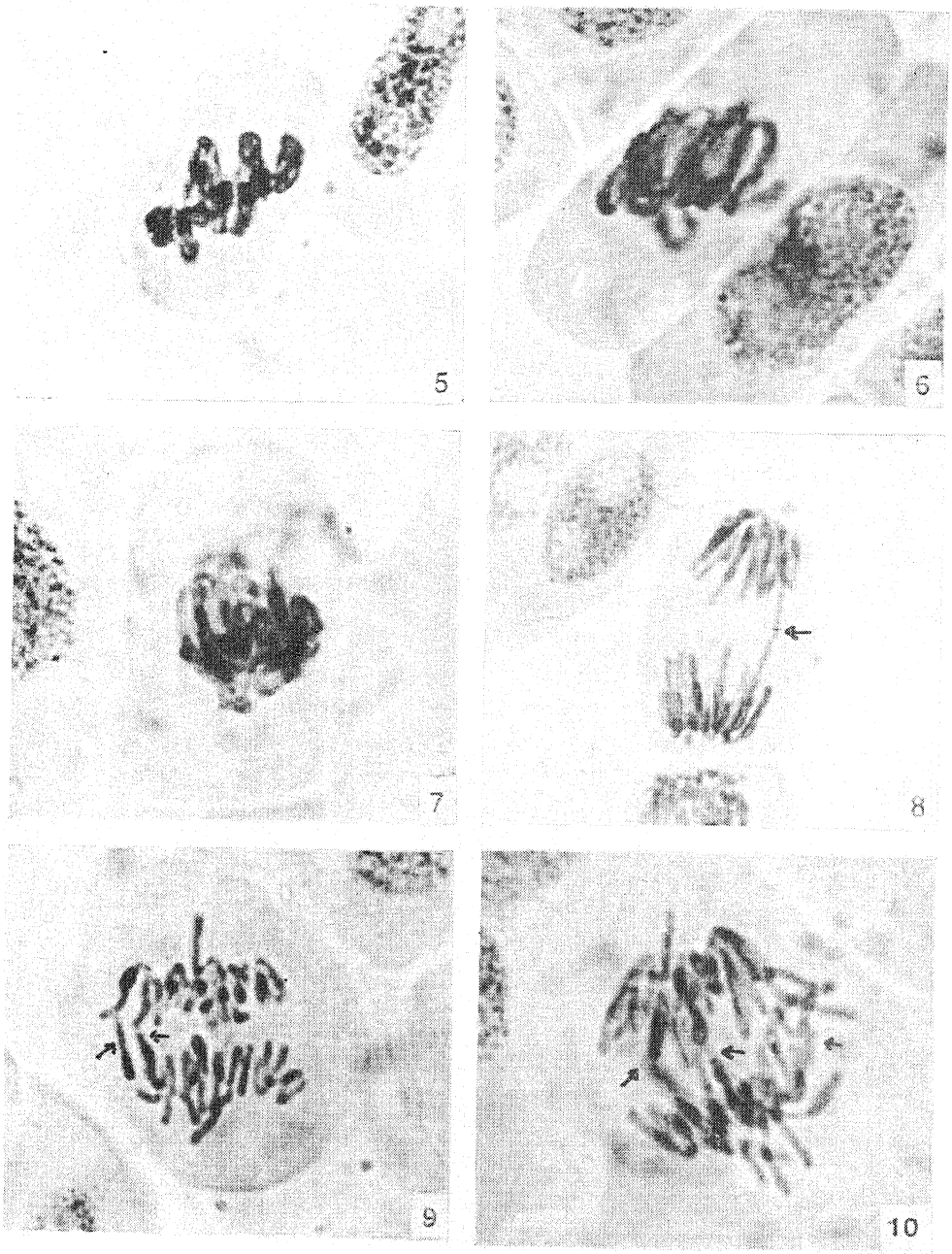

Fig. 5, 6– Stickiness. Fig. 7– Clumping. Fig. 8– Single bridge at anaphase.
Fig. 9– Double bridge at anaphase. Fig. 10– Multiple bridge at anaphase.

Table 1– Mitotic index and percent frequency of abnormality and DR in pesticide treated root tips of *Allium cepa*

Treatments (%)	No of cells observed	No of dividing cells	Mitotic index	No of abnormal cells	RDR	RAR
Control	530	109	20 57 ± 0 07			
Aldrin						
0 1	490	87	17 76 ± 0 17	13 ± 0 63	- 3 54 ± 0 06	2 65 ± 0 05
0 2	511	82	16 05 ± 0 03	15 ± 0 39	- 5 69 ± 0 08	2 94 ± 0 06
0 3	500	72	14 40 ± 0 02	19 ± 0 28	- 7 77 ± 0 09	3 80 ± 0 05
0 4	467	58	12 42 ± 0 03	15 ± 0 79	- 10 26 ± 0 10	3 21 ± 0 03
0 5	500	55	11 00 ± 0 03	19 ± 0 49	-12 05 ± 0 11	3 80 ± 0 08
1 0	526	48	9 13 ± 0 02	26 ± 0 56	- 14 40 ± 0 12	4 94 ± 0 10
Malathion						
0 1	477	81	16 98 ± 0 09	14 ± 0 48	- 4 52 ± 0 09	2 94 ± 0 08
0 2	494	76	15.38 ± 0.11	17 ± 0 56	- 6 53 ± 0 10	3 44 ± 0 10
0 3	508	69	13 58 ± 0 03	20 ± 0 74	- 8 80 ± 0 07	3 94 ± 0 07
0 4	473	48	10 15 ± 0 05	22 ± 0 49	- 13 12 ± 0 09	4 65 ± 0 11
0 5	414	37	8 94 ± 0 05	19 ± 0 74	- 14 64 ± 0 07	4 59 ± 0 09
1 0	511	41	8 02 ± 0 05	29 ± 0 28	-15 80 ± 0 07	5 68 ± 0 10
Monocrotophos						
0 1	468	75	16 03 ± 0 09	15 ± 0 74	- 5 72 ± 0 06	3 21 ± 0 13
0 2	495	65	13 13 ± 0 12	20 ± 0 28	- 9 37 ± 0 07	4 04 ± 0 08
0 3	455	53	11 65 ± 0 10	23 ± 0 40	- 11 23 ± 0 09	5 06 ± 0 13
0 4	517	44	8 51 ± 0 13	28 ± 0 74	- 15 18 ± 0 07	5 42 ± 0 18
0 5	466	38	8 15 ± 0 03	28 ± 0 40	- 15 64 ± 0 07	6 01 ± 0 17
1 0	471	35	7 43 ± 0 10	29 ± 0 63	- 16 54 ± 0 08	6 16 ± 0 14

ɔle 2– Mitotic analysis in RTCs of *Allium cepa* following treatment with different concentrations

eatment (%)	TRTCs	Clastogenic aberrations				%AbC (a)	Physiological aberrations				%AbC (b)	% TAbC (a+b)
		ChFr (%)	Rc (%)	ChBr (%)	MN (%)		ST (%)	LgCh (%)	MP (%)	DNC (%)		
ntrol	530											
drin												
0 1	490	0 41 ±0 03	-	0 41 ± 0 05	0 41 ± 0 06	1 23 ± 0 09	0 61 ± 0 08	0 61 ± 0 06	0 20 ± 0 05	-	1 42 ± 0 06	2 65 ± 0 05
0 2	511	0 59 ± 0.02	-	0 59 ± 0 04	0 39 ± 0 05	1 57 ± 0 11	0 59± 0 04	0 78 ± 0 06	-	-	1 3 7 ± 0 07	2 94 ± 0 06
0 3	500	0 60 ± 0 06	-	0 80 ± 0 05	0 60 ± 0 06	2 00 ± 0 12	0 60± 0 04	1 00 ± 0 04	0 20 ± 0 05	-	1 80 ± 0 09	3 80 ± 0 05
0 4	467	0 64 ± 0 05	-	0 86 ± 0 03	0 43 ± 0 07	1 93 ± 0 10	0 2 1±0 03	0 86 ± 0 05	0.21 ± 0 04	-	1 28 ± 0 06	3 21 ± 0 03
0 5	500	0 60 ± 0 06	0 20 ± 0 05	1 00 ± 0 07	0 60 ± 0 09	2 40 ± 0 10	0 40± 0 03	1 00 ± 0 04	-	-	1 40 ± 0 04	3 80 ± 0 08
1 0	526	0 76 ± 0 04	0 38 ± 0 06	1 14 ± 0 05	0 76 ± 0 07	3 04 ± 0 07	0 38± 0 03	1 14 ± 0 05	0 19± 0 03	0 19 ± 0 05	1 90 ± 0 06	4 94± 0.10
ılathıon												
0 1	477	0 42 ± 0 06	-	0 63 ± 0 09	0 21 ± 0 03	1 26 ± 0 10	0 63± 0.04	0 63 ± 0 06	0 42 ± 0 06	-	1 68 ± 0 07	2 94 ± 0 08
0 2	494	0 61 ± 0 08	-	0 81 ± 0 07	0 20 ± 0 05	1 62 ± 0 11	0 81±0 03	0 81 ± 0 04	0 20 ± 0 04	-	1 82 ± 0 06	3 44 ± 0 10
0 3	508	0 59 ± 0 07	-	0 98 ± 0 06	0 20 ± 0 05	1 77 ± 0 08	0 79± 0 04	0 98 ± 0 04	0 20 ± 0 04	0 20 ± 0 05	2 17 ± 0 04	3 94± 0 07
0 4	473	0 63 ± 0 08	0 21 ± 0 04	1 06 ± 0 05	0 21 ± 0 05	2 11 ± 0 09	0 85± 0 05	1 06 ± 0 05	0 21 ± 0 04	0 42 ± 0 05	2 54 ± 0 09	4 65 ± 0 11
0 5	414	0 73 ± 0 05	0 24 ± 0 06	1 21 ± 0 07	0 24 ± 0 03	2 42 ± 0 11	0 48± 0 06	1 21 ± 0 04	0 24 ± 0 05	0 24 ± 0 04	2 17 ± 0 05	4 59 ± 0 09
1 0	511	0 98 ± 0 06	0 39 ± 0 06	1 37 ± 0 06	0 39 ± 0 04	3 13± 0 10	0 39± 0 04	1 18 ± 0 04	0 59 ± 0 08	0 39 ± 0 05	2 55 ± 0 07	5 68 ± 0 10
ɔnocrotophos												
0.1	468	0 43 ± 0.05	-	0 86 ± 0 08	0 21 ± 0 05	1 50 ± 0 10	0 64± 0 05	0 64 ± 0 06	0 43 ± 0 03	-	1 71 ± 0 04	3 21± 0 13
0 2	495	0 61 ± 0 08	-	1 01 ± 0 07	0 20 ± 0 04	1 82 ± 0 12	0 81± 0 04	0 81 ± 0 06	0 40 ±0 04	0 20 ± 0 03	2 22 ± 0 06	4 04 ± 0 08
0 3	455	0 88 ± 0 06	-	1 10 ± 0 06	0 44 ± 0 04	2 42 ± 0 09	0 88± 0 06	1 10 ± 0 04	0 44 ± 0 06	0 22 ± 0 03	2 64 ± 0 06	5 06 ± 0 13
0 4	517	0 97 ± 0 07	0.19 ± 0 04	1 16 ± 0 07	0 58 ± 0 03	2 90± 0 10	0 97± 0 05	0 97 ± 0 05	0 39 ± 0 05	0 19 ± 0 04	2 52 ± 0 06	5 42 ± 0 18
0 5	466	1 07 ± 0 08	0 22 ± 0 06	1 29 ± 0 06	0 64 ± 0 04	3 22± 0 10	1 07± 0 05	1 07 ± 0 04	0 43 ± 0 06	0 22 ± 0 04	2 79 ± 0 09	6 01± 0 17
1 0	471	1 06 ± 0 07	0 21 ± 0 05	1 49 ± 0 07	0 64 ± 0 05	3 40 ± 0 08	0 80± 0 06	1 27 ± 0 06	0 43 ± 0 06	0 21 ± 0 05	2 76 ± 0 09	6 16± 0 14

'Cs - Total tıp cells observed, ChFr - Chromosome fragments, Rc - Rıng chromosomes, ChBr - Chromatın brıdges, MN - Mıcronuclei %AbC - Percent aberrant cells ST - Stickiness, h - Lagging chromosomes, MP - Multipolarity, DNC - Dinucleate cells, % TAbC - Percent total aberrant cells

Ring chromosomes were not observed at lower concentrations of the pesticides but at higher concentrations these were present. Maximum percentage of ring chromosomes was observed (0.39 ± 0.06%) at 1.0% malathion treatment and minimum percentage (0.19 ± 0.04%) was observed at 0.4% monocrotophos treatment.

The most prominent abnormality at the anaphase was the formation of the chromatin bridges (single, double and multiple bridges). Chromatin bridges were prominent at all the treatment. The frequency of cells with bridges ranged from 0.41 ± 0.05 - 1.14 ± 0.05%, 0.63 ± 0.09 - 1.37 ± 0.06% and 0.86 ± 0.08 - 1.49 ± 0.07% during the treatments of aldrin, malathion and monocrotophos, respectively.

Micronuclei were also observed at all the treatment doses of pesticides. The micronuclei show maximum frequency (0.76 ± 0.07%) at 1.0% aldrin treatment and the minimum (0.20 ± 0.05 and 0.20 ± 0.04%) at 0.2% and 0.3% concentrations of malathion and 0.2% concentration of monocrotophos.

The percentage of stickiness was gradually increased with the increase of concentrations of pesticides but decreased at very high concentrations. Its frequency ranged from 0.21 ± 0.03 0.61 ± 0.08%, 0.39 ± 0.04 - 0.85 ± 0.05% and 0.64 ± 0.05 - 1.07 ± 0.05% in the different treatments of three pesticides, respectively. The maximum frequency (1.07 ± 0.05%)was observed at 0.5% monocrotophos treatment and the minimum 0.21 ± 0.03 at 0.4% aldrin treatment.

Laggards were commonly observed at anaphase in all the treatments. The maximum percentage of laggards (1.27 ± 0.06%) was observed at 1.0% monocrotophos treatment, and the minimum (0.61 ± 0.06%) at 0.1% aldrin treatment.

Multipolarity was not found in a proper manner. The highest percentage (0.59 ± 0.08%) was observed at 1.0% malathion treatment and lowest percentage (0.19 ± 0.03%) at 1.0% aldrin treatment.

Dinucleate cells were not observed at low concentrations of the pesticides but found at higher concentrations. The maximum percentage (0.39 ± 0.05%) was observed at 1.0% malathion treatment and minimum (0.19 ± 0.05 and 0.19 ± 0.04%) at 1.0% aldrin and 0.4% monocrotophos.

Discussion

All the three pesticides used in the present study were found to have lethal effect on cell division-and induced cytological disturbances during root tip mitosis. All the

concentrations were capable of inducing different types of chromosomal abnormalities (Figs. 4-9) and the frequency of abnormalities increased in most of the cases along with increase in pesticide concentration. Similar type of genotoxic effects of different chemicals have already been carried out on *Vicia faba, Allium cepa, Trigonella foenum-graecum* and legumes by several workers[3,4,5,6,7]. The breakage of chromosomes is now generally considered to involve DNA molecule, which is responsible for the linear continuity of the chromosome and may be due to unfinished or misrepair of DNA[8]. Alterations in the fidelity of replication of normal DNA and nucleotide pools may be the other cause of chromosomal aberrations. It is logical to assume that the pesticides might have interfered with the normal functions of repair enzymes and series of reactions were involved in the process of rejoining or altering the nucleotide pool.

Stickiness of chromosomes observed in this study can be interpreted as the interrelation of the pesticides with DNA leading to entanglement of chromatin threads[9]. The chromosomal bridges observed during anaphase might be due to chromosomal stickiness, unequal translocation or inversion of chromosome segments followed by crossing over: Micronuclei observed during the treatment of all the three pesticides are resultants of clastogenic events of the cells concerned rather than mitoclastic events[10]. Formation of binucleate/multinucleate cells was noticed that the pesticides inhibited the cell plate formation.

The study reveals that the pesticides exerted wide range of clastogenic as well as turbagenic effects similar to many other pesticides. From the present test, it appears that aldrin, malathion and monocrotophos, which are used frequently at kitchen garden in addition to agriculture field, have positive chromotoxic effects. They can affect .fertility and may alter the genetic constitution of the plants by causing chromosomal abnormalities. Present investigation thus, warrants excessive and indiscriminate spraying of these chemicals for plant protective measures at kitchen garden and agriculture field.

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